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EDITED BY

W. H. PEARSALL
D.Sc., F.R.S.
Quain Professor of Botany
University College, London

ASSISTED BY

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Emeritus Professor of Botany
The University, Cambridge

D. G. CATCHESIDE, D.Sc.
The Botany School, Cambridge

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Professor of Botany
The University, Reading

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Experimental and Analytical Studies of Pteridophytes

XVI. The Induction of Leaves and Buds in *Dryopteris Aristata* Druce

BY

C. W. WARDLAW

(*Department of Cryptogamic Botany, University of Manchester*)

With Plates XV and XVI and twenty Figures in the Text

I. INTRODUCTION

BY appropriate treatments of the shoot apex of *Dryopteris aristata* Druce, leaf primordia can be induced to arise in positions other than their normal presumptive positions, buds can be induced in what are normally leaf positions, and leaf primordia in bud positions. These experimental data are held to support the hypotheses that the positions in which lateral members arise at the shoot apex and the regulated development of the leafy shoot can be referred to the existence of growth centres and their physiological fields (Wardlaw, 1949*c*). The underlying ideas, based on the work of Schoute (1913), are these: (i) that the extreme apex of the shoot, and also each young leaf primordium, constitutes a growth centre with a surrounding physiological field; (ii) that new growth centres can only originate outside the existing fields; and (iii) that no fundamental metabolic differences exist between shoot and leaf apices, though there may be differences in the extent or intensity of their fields. By isolating particular regions of the apical meristem by vertical incisions, primordia of large relative size are induced, i.e. inhibitory effects of adjacent primordia have been precluded. A result of particular interest is that leaf primordia can be induced experimentally, the induced leaves arising either on the bases of existing primordia of which the apices had been damaged, or in interfoliar, i.e. in bud, positions.

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These observations open up a large and interesting field for further investigation. In the present paper further observations on the induction of leaves and buds are discussed in relation to the hypotheses set out above.

II. Methods and Materials

Large apices of *Dryopteris aristata* Druce were prepared for experimental treatment as already described (Wardlaw, 1947, 1949*c*). Small incisions were made in apices as required by means of fine knives. The apices were washed, placed in moist peat, and kept under observation. From embedded materials structural and histological data were obtained. The semi-diagrammatic drawings in the text were made by means of a camera lucida.

Terminology. The terminology used to indicate existing primordia P_1, P_2, P_3 (in order of increasing age) and primordia yet to be formed, I_1, I_2, &c. (in the order of their appearance) is that of M. and R. Snow (1931). I_1, I_2, &c., are also used to indicate primordia which in due course appeared in the experimental materials.

III. Experimental Results

(*a*) *The effect of puncturing* P_1–P_5 *and* I_1–I_3

The basis of this experiment may be briefly explained. In *Dryopteris*, bud positions occur on the shoot a little above the leaf axil, Text-fig. 1; they are more aptly described as being interfoliar. It will be seen that some bud positions occur on the apical meristem at about the same level as the top cycle of leaf primordia (Text-fig. 1), but in the normal development bud formation in these positions is inhibited. On the hypotheses under consideration, the inhibition of buds at the apex may be due in part to the shoot apex but chiefly to the adjacent leaf primordia. It was therefore argued that if (i) the apices of primordia P_1–P_5 were punctured, (ii) the positions I_1–I_3 were punctured or incised so that the development of their physiological fields would be precluded, and (iii) the apical cell was still inactive, as at the beginning of the growing season, conditions would have been established which would admit of the formation of lateral organs in the normal bud positions on the apical meristem. The appearance of such an experimental apex is illustrated in Text-fig. 2. An initial series of experiments carried out along these lines has already been described (Wardlaw, 1949, 1949*c*): the first indication of growth, apart from the formation of scales, was the appearance of small outgrowths on the punctured primordia P_1–P_5, or in interfoliar positions, lateral to P_1–P_5, or axillary to P_6–P_{10} (Text-fig. 3). These small outgrowths, on further development, were seen to be leaf primordia. Some of them occupied what are normally bud positions.

These experiments were undertaken from February onwards, i.e. prior to and at the beginning of the normal growing season for this fern, when the region of the apical cell is still quiescent. When the experiment was repeated, using apices in groups of five, at monthly intervals from April until July, results closely comparable with those in Text-fig. 3 were consistently obtained.

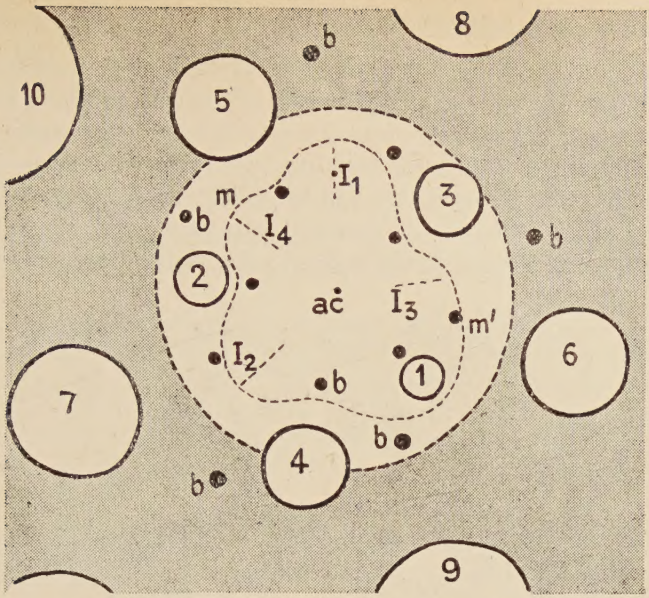


FIG. 1. Apex of *Dryopteris aristata* as seen from above, showing the top cycle of leaf primordia (*P*) 1–5 and the older leaf primordia 6–10; I_1, I_2, and I_3 indicate the next leaf primordia to be formed in the order of their appearance; *b*, the positions of bud rudiments, i.e. bud sites; *m–m′* indicates the lower limit of the superficial, prismatic meristematic cells which, together with the apical cell (*ac*), constitute the *apical meristem*. The circular broken line represents approximately the base of the apical cone and the beginning of the broad subapical region. (Diagrammatic × 30.)

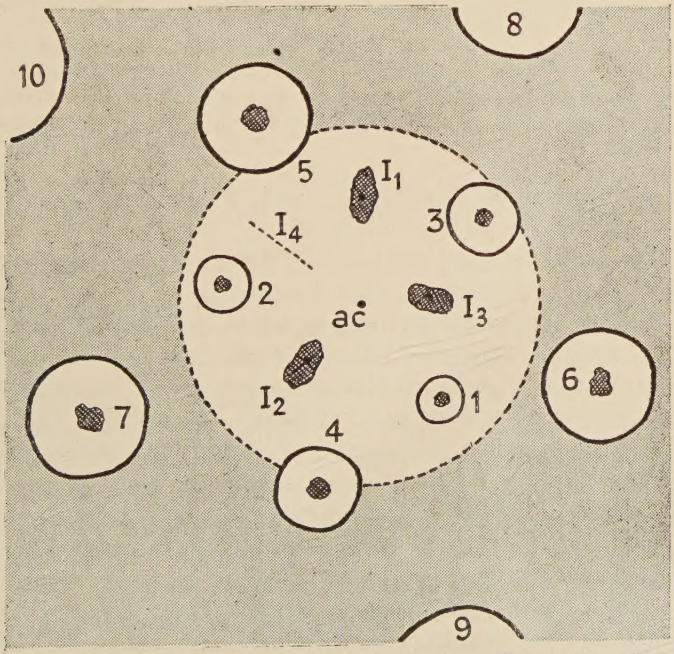


FIG. 2. An experimental apex: the apices of leaf primordia 1–5 have been punctured and also the positions I_1, I_2, and I_3. The position of I_4 is indicated; *ac*, apical cell. (Diagrammatic × 30.) (In the illustrations in this paper, punctured or damaged tissue is cross-hatched.)

The inhibition, in the normal development, of the formation of lateral members between the top cycle of leaf primordia is due, therefore, to the adjacent leaf primordia and not apparently to the extreme tip of the shoot.

In the foregoing experiments both the top cycle of existing primordia, P_1–P_5, and the cycle of primordia yet to appear, I_1–I_3, or I_1–I_4, had been punctured. Further experiments were therefore required to ascertain the part played by these two groups in the normal inhibition of interfoliar members.

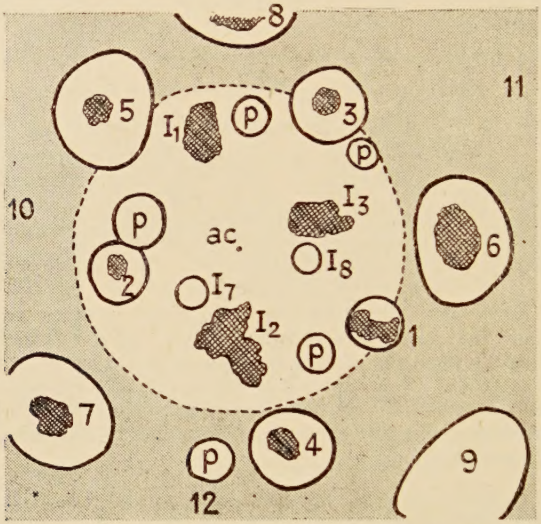


FIG. 3. An apex treated as in Fig. 2, after further growth. Interfoliar leaf primordia (*p*) have been induced between the leaf primordia of the top cycle, i.e. P_1–P_5; some of these induced primordia are axillary to older leaf primordia, e.g. P_8–P_{12}. No new primordia have appeared in the normal I_4, I_5, or I_6 positions, but I_7 and I_8 occupy approximately normal positions. (×27.)

The results of incising P_1–P_5 only, and of I_1–I_4 only, are described in the following sections.

Note. In the writer's view, the essential differences in the morphology of leaves and lateral shoots in ferns, i.e. dorsiventral as compared with radial symmetry, &c., are due to the positions in which they are formed. Since leaf primordia are always formed on the apical meristem, i.e. on the sides of a cone, with a considerably greater rate of growth on the abaxial as compared with the adaxial side, and with possible inhibitional effects proceeding from the apical cell on the adaxial side, the dorsiventral symmetry of leaves appears as a natural consequence. Buds, on the other hand, are typically formed in older, mature regions of the shoot, where this asymmetry of position does not obtain, and they characteristically develop with radial symmetry. If, now, lateral organs are induced to develop in what are normally bud positions on the apical cone about the level of the top cycle of primordia, i.e. P_1–P_5, these organs, for the reasons given above, will have dorsiventral symmetry and will be recognized as foliar members. In this view, leaves and lateral shoots are

not specifically determined either by the hereditary constitution of the organism or by the pre-existence in the race of organs of different fundamental categories (Wardlaw, 1949*c*).

(*b*) *The effect of puncturing* P_1–P_5

In several series of experiments primordia P_1–P_5 were punctured and positions I_1–I_4 left intact. In Text-fig. 4 a typical result is illustrated. After some

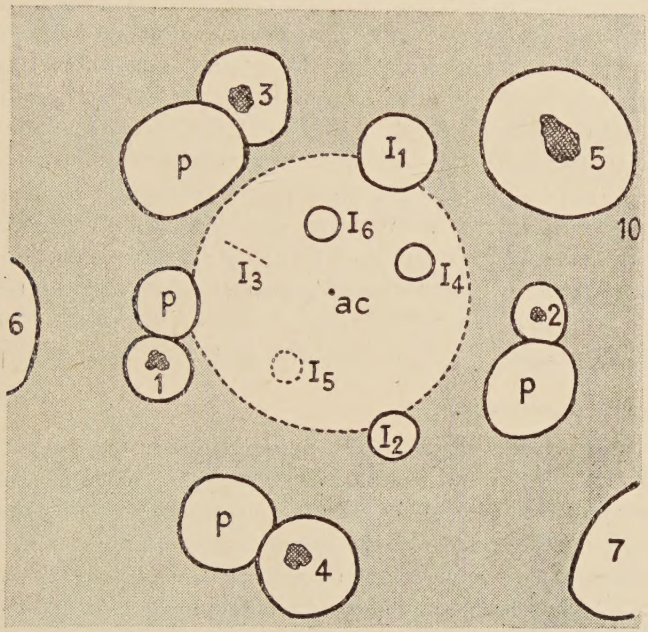


FIG. 4. An apex in which P_1–P_5 were punctured: interfoliar primordia (*p*) have been induced; I_1 and I_2 have appeared in their normal positions; there is no primordium in the I_3 position, but one has formed at I_4; in a later examination the primordium I_5 was observed, in normal phyllotactic sequence with I_4. (×27.)

time induced leaf primordia could be observed close to or associated with the bases of P_1, P_2, P_3, and P_4. The primordia lateral to P_1 and P_2 are axillary to P_6 and P_7 respectively. The new primordia I_1 and I_2 appeared in their normal positions. On further growth the induced primordia grew rapidly and tended to obliterate the original primordia P_1–P_4. No primordium was formed in the I_3 position presumably because of the presence of the induced primordium axillary to P_6 and lateral to P_3 in that sector. The next primordium to appear, and labelled I_4, is in the normal position for that primordium. The next again, labelled I_6, arose in the sector P_1–I_1, i.e. no primordium had yet appeared in the normal I_5 position in the P_1–I_2 sector. But on further growth a primordium, labelled I_5, appeared in the I_5 position. The position of this primordium is normally determined by I_2 and I_3.

Some apices showed considerably greater growth activity than others. Text-figs. 5 and 6 illustrate apices in which I_1–I_4 were formed after P_1–P_5 had been punctured. In each apex only one induced leaf-base primordium was formed (on the base of P_4, Text-fig. 5, and P_2 in Text-fig. 6). But in each apex an induced interfoliar primordium appeared axillary to P_6, more or less contemporaneously with the formation of I_3, so that the two primordia were observed side by side as illustrated. In other words, an induced primordium arose in that sector of the apical meristem which remained longest without a new normal primordium (i.e. I_3). If I_1 and I_2 are soon formed in their respective positions, and assuming that they exercise a lateral and basipetal inhibitional effect, we should not expect interfoliar primordia axillary to P_8 and P_7 to arise. But as a longer time will normally elapse before I_3 is formed and since, with the continued growth of the apex, the sector P_1–P_3 is expanding tangentially all the time, the induction of a primordium in the interfoliar position P_1–P_3, i.e. axillary to P_6, is what might be expected. Considerations such as these would appear to afford an interpretation of the data of Text-figs. 5 and 6. Several observations of this kind have been made.

The general result of this series of experiments is that the inhibition of interfoliar primordia is due initially and mainly to primordia P_1–P_5, but the next cycle of primordia, i.e. I_1–I_3, may also have some part in this activity.

(*c*) *The effect of puncturing* I_1–I_3

In this series the positions of I_1–I_3 were punctured or incised and primordia P_1–P_5 left intact. If the primordia arising at I_1–I_3 normally exercised an inhibitory effect on the interfoliar primordia of the cycle P_1–P_5, then, in these experiments, by precluding the development of the inhibitory fields of I_1–I_3, some induction of primordia might be expected, especially when the interfoliar spaces have become extended tangentially during growth. Furthermore, the new primordia subsequently appearing on the meristem, i.e. I_4, I_5, and I_6, might be expected to arise close to the adjacent wounds, i.e. those in the I_1 and I_2 positions (Wardlaw, 1949*b*, 1949*c*).

Text-fig. 7 illustrates an apex 4 weeks from the beginning of the experiment. The positions I_1–I_3 were incised but no new primordia, normal or induced, have yet appeared. (Primordium P_4 was injured during one of the periodic examinations and a lateral primordium is beginning to appear.) Text-fig. 8 illustrates a rapidly growing apex after 4 weeks: no interfoliar primordia have been induced in the cycle P_1–P_5, but new primordia have arisen on the meristem above. These constitute part of the normal sequence, i.e. I_4, I_5, and I_6, but all have been formed close to the adjacent wounds (I_1 and I_2). The normal positions of I_4–I_6 are indicated by broken lines. This is the kind of record that has been obtained in eight apices out of ten in this experiment. In Text-fig. 9, I_4 and I_5 were formed close to the I_1 and I_2 incisions respectively, but I_6 occupies a position just above the I_3 incision. In the untreated control apices, two to five new primordia, i.e. I_1–I_5, had been formed during this period, but no interfoliar primordia appeared. In another experiment of

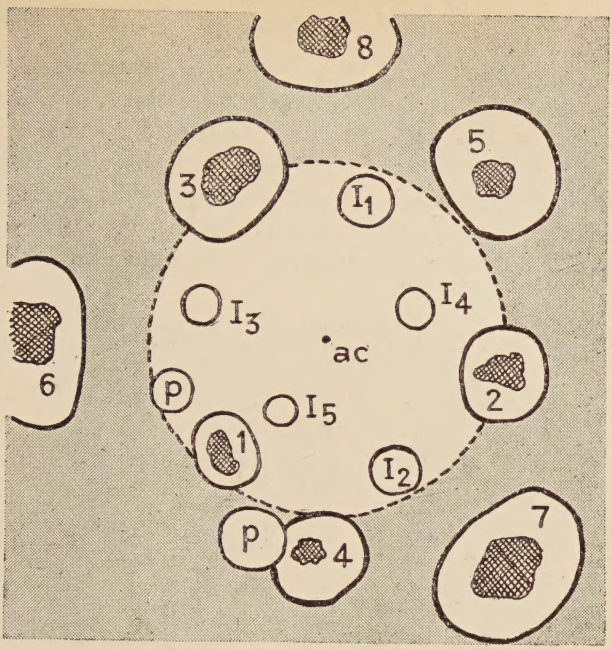


FIG. 5. Experimental treatment as in Fig. 4. An induced interfoliar leaf primordium (p), lateral to P_1, and the new primordium at I_3 have been formed contemporaneously in the same space. (×27.)

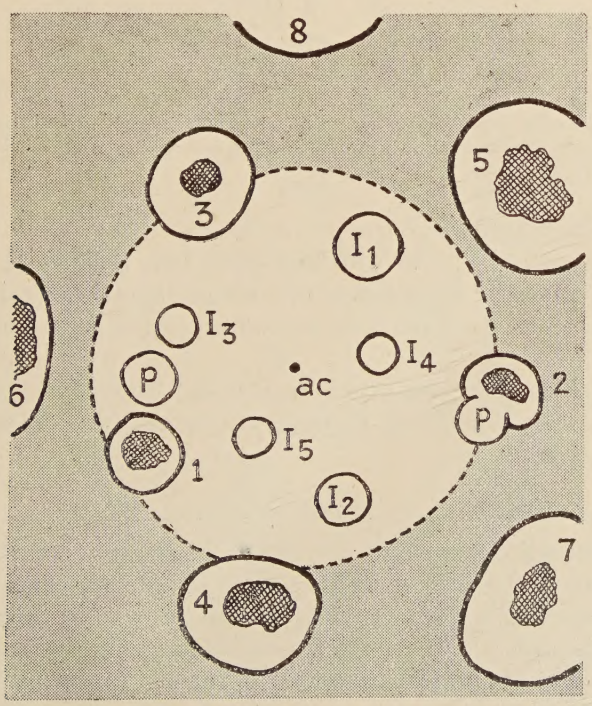


FIG. 6. Another record comparable to that illustrated in Fig. 5. In this apex new primordia have appeared in rapid succession on the apical meristem and only two interfoliar primordia (p) have been induced. (×27.)

this series, in which positions I_1–I_3 were incised, interfoliar primordia were induced in the axils of P_6, P_7, and P_8 in three apices out of five. In these apices primordia only arose at I_4, I_5, and I_6 after a considerable lapse of time, i.e. the meristem was rather inactive. It thus appears that while primordia P_1–P_5 normally inhibit the formation of interfoliar primordia during the first 2–4 weeks, interfoliar members may be induced as the gaps widen during growth, if new primordia are not formed on the meristem above. In the normal

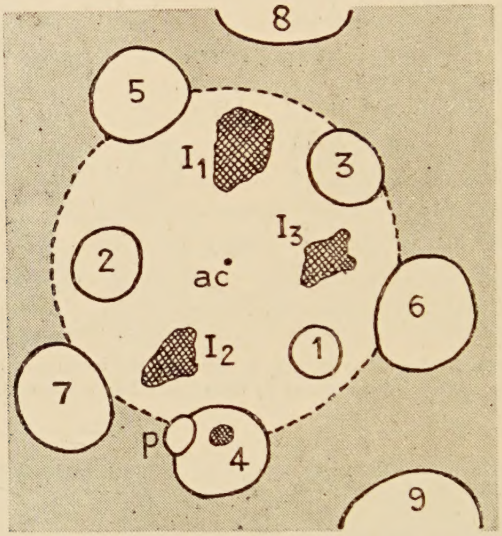


FIG. 7. An apex in which the I_1, I_2, and I_3 positions were punctured, P_1–P_5 being left intact. After 4 weeks, when this record was made, no induced interfoliar primordia had appeared (at P_4, which was injured during one of the inspections, a primordium (*p*) is beginning to form). (×27.)

development new primordia do appear and their fields preclude the formation of interfoliar primordia in the widening gaps of the cycle P_1–P_5. It is evident that the part played by the apical cell (or the group of cells at the extreme tip of the shoot) must be taken into account in these experiments (see next section).

The general result of these experiments is that where the apex is active and the apical cell and primordia P_1–P_5 intact, interfoliar primordia are not induced in the cycle P_1–P_5.

(*d*) *The effect of puncturing* P_1–P_5 *and the apical cell*

If the apical cell is punctured, leaf primordia continue to be formed on the meristem until the available space is used up, and buds, axillary to some of the older leaf primordia, are also formed (Wardlaw, 1949*a*, *b*). Also, if a tangential incision is made just above the I_1 position, a bud arises there, i.e. in what is normally a leaf position (Wardlaw, 1949, 1949*c*). In the first instance mentioned above, the apex undergoes further growth: parenchymatous tissue

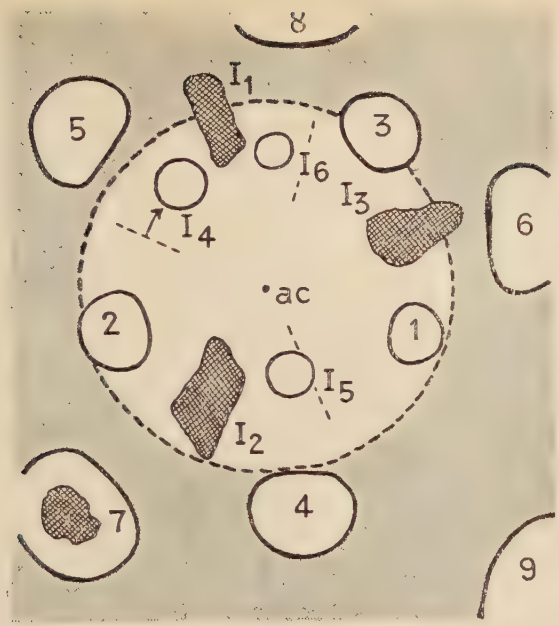


FIG. 8. Experimental treatment as in Fig. 7: no interfoliar primordia have been induced and I_4, I_5, and I_6 show characteristic displacement towards the wounds at I_1 and I_2. (×27.)

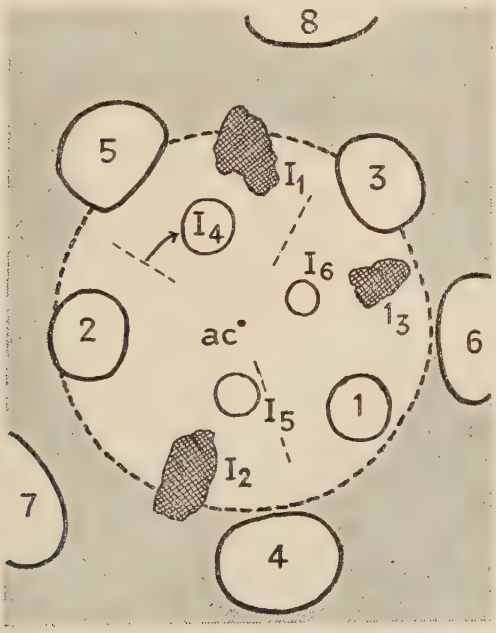


FIG. 9. Experimental treatment as in Figs. 7 and 8: no induced interfoliar primordia have appeared; I_4 and I_5 have appeared close to the wounds at positions I_1 and I_2, but I_6 occupies a position between P_1 and I_4. (×27.)

develops in the necrosed apical cell region, the subapical region enlarges, and what was originally the apical cone becomes somewhat flattened out. It is from superficial meristematic cells on this flattened region that buds arise.

In view of the results set out in the preceding sections, it was a matter of interest to ascertain what would happen if the shoot apical cell and the apices of P_1–P_5 were punctured at the same time. Some of the records obtained are illustrated and described below.

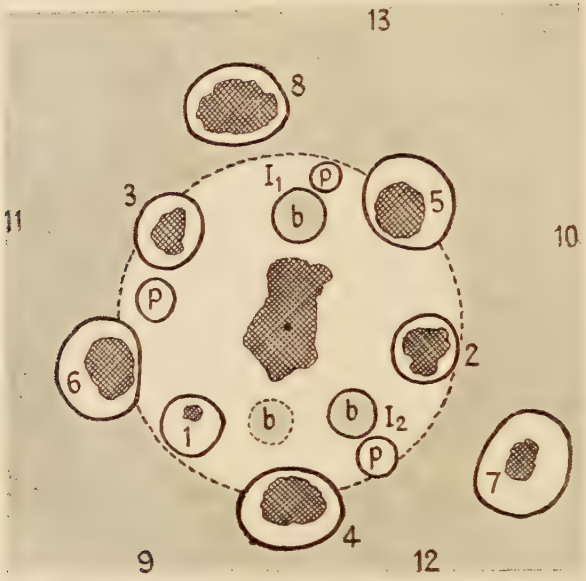


FIG. 10. An apex in which primordia P_1–P_5 have been punctured and also the apical cell. Interfoliar primordia (*p*) have been induced above the axils of P_{11}, P_{12}, and P_{13}; buds (*b*) have appeared in the I_1 and I_2 positions and above the axil of P_4. (×27.)

In the apex illustrated in Text-fig. 10, the first evidence of primordium formation was in the cycle of P_1–P_5; these were leaf primordia and could be interpreted as lying above the axils of P_{11}, P_{12}, and P_{13}. Soon after, shoot buds were observed in the I_1 and I_2 positions and later a further shoot bud was observed above the axil of P_4. In Text-fig. 11, interfoliar primordia have appeared in association with P_2, P_3, and P_4; and whereas a leaf primordium has been formed at I_1, a bud has arisen in the I_2 position; a bud has also been formed in the axil of P_6. In Text-fig. 12 the position of I_1 was damaged and has become necrosed, various interfoliar leaf primordia (*p*) have been induced, and buds have been formed in approximately the I_2 and I_3 positions (or perhaps they are axillary to P_7 and P_6 respectively); the primordium in the axil of P_5 was also a bud. Text-fig. 13 also shows various induced interfoliar leaf primordia (*p*), a bud with its first leaf primordium in the normal I_1 position, a leaf primordium in the normal I_2 position, a bud in the I_3 position (or perhaps it is axillary to P_6), and a bud basal to P_1. Text-figs. 14 and 15 show the same apex on two successive weeks. Induced interfoliar leaf primordia are

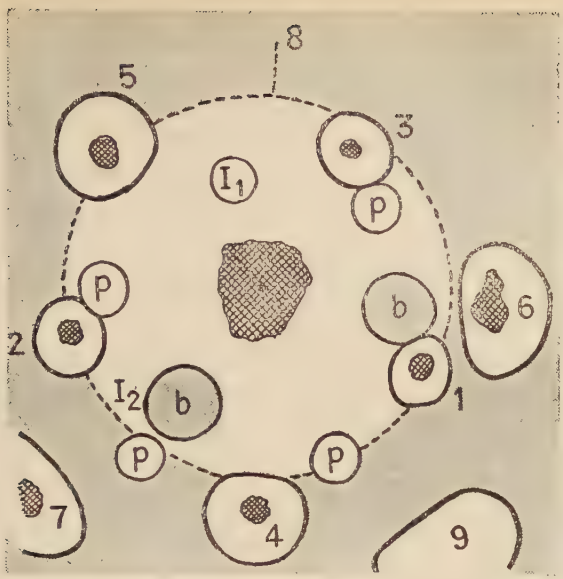


FIG. 11. Experimental treatment as in Fig. 10: some interfoliar primordia (*p*) have been induced; and whereas a leaf primordium has been formed in the I_1 position, a bud (*b*) has been formed in the I_2 position; a bud (*b*) was also formed subsequently above the axil of P_6. (×27.)

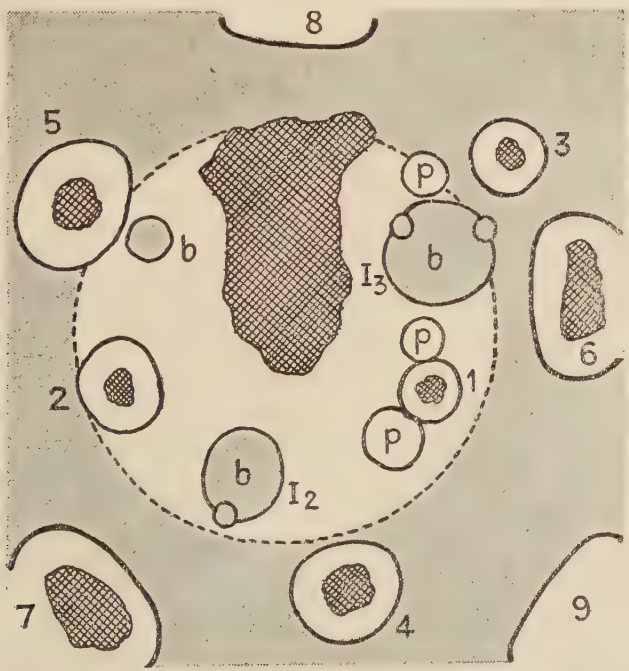


FIG. 12. Experimental treatment as in Fig. 11, the I_1 position being also damaged and necrosed. Induced leaf primordia (*p*) and buds (*b*) with their first leaves have appeared. (×27.)

associated with P_1 and P_2 (Text-fig. 14), a leaf primordium has appeared at I_1, a bud close to the I_2 position, and a leaf primordium at I_3. In Text-fig. 15 these observations are confirmed, but a bud has now arisen above the axil of I_1 and another lateral to P_1 and roughly above the axil of P_6.

At this stage it is not possible to offer any adequate explanation of these several organogenic developments. The induction of interfoliar primordia in

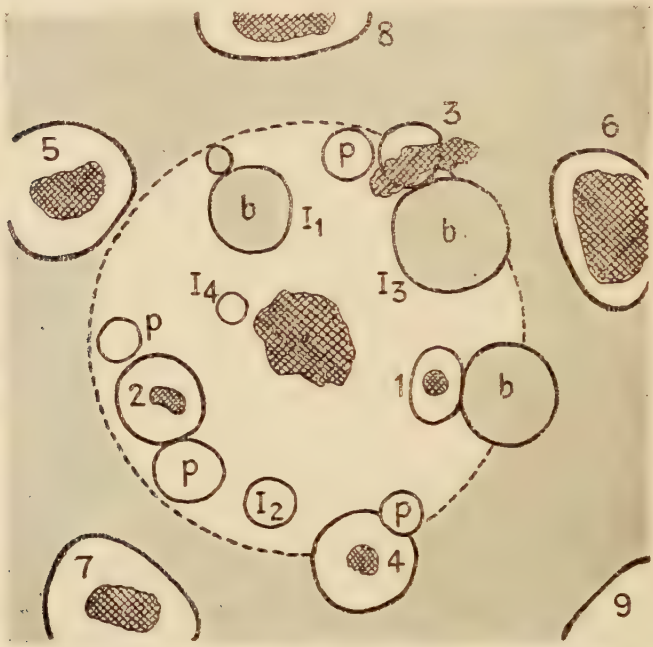


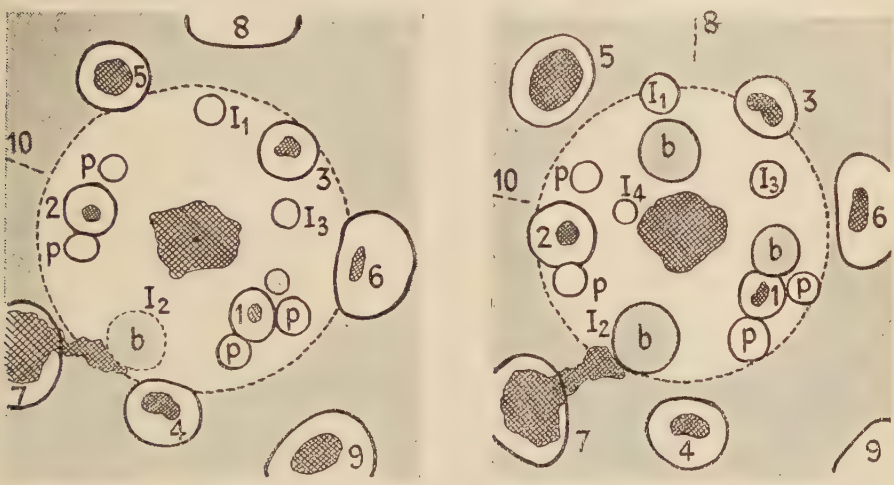
Fig. 13. Experimental treatment as in Figs. 11 and 12: the I_1 position is occupied by a bud (*b*) with its first leaf, the I_2 and I_4 positions by leaf primordia, and the I_3 position (approximately) by a bud; induced interfoliar primordia (*p*) have also appeared. (×27.)

the cycle of P_1–P_5 is in agreement with the findings already set out in section III (*a*). The formation of buds in what are normally leaf positions (i.e. at I_1, I_2, or I_3) when the action of the apical cell is removed or precluded has also been the subject of comment (Wardlaw, 1949, 1949*c*). But two features in these records do present special problems. One is that two growth centres, whether of leaf or bud primordia, can apparently originate close together or fairly close together, contemporaneously. The other is that, on the same apex, whereas at one presumptive leaf position a leaf primordium may arise, as in the normal development, at another presumptive leaf position it is a bud primordium that is formed. From this it may be inferred that, in the ferns, quite small differences in the incidence of morphogenetic factors in the apical meristem may determine whether it is a leaf or a bud primordium that is formed. Since the developments under consideration have been obtained when the apical cell was punctured, with concomitant disruption of the

normal conical construction, it may be tentatively suggested that physical factors and factors determining the distribution of growth are involved.

(*e*) *Minimal space for the formation of a leaf primordium*

According to M. and R. Snow (1931, 1947) a leaf will be formed in the 'next available space' on the apical meristem that attains a certain necessary width and a certain minimal distance below the extreme tip of the shoot. Now, in the ferns, as Text-fig. 1 clearly shows, a new primordium occupies



FIGS. 14 and 15. Experimental treatment as in Fig. 13: the same apex is shown on two successive weeks. Various interfoliar primordia (*p*) have been induced; leaf primordia have appeared in the I_1, I_3, and I_4 positions and buds (*b*) in the I_2 position and above the axils of I_1 and P_6 (approx). (×20.)

only a small part of the area between two adjacent primordia. Primordium P_1, for example, occupies only a small part of the space between P_4 and P_6. If space on the meristem has the importance attributed to it in the matter of leaf formation, then by curtailing the space between P_3 and P_5, or any other pair of adjacent primordia, the formation of a primordium in that space should be precluded.

In the course of these studies, in which the apex has been incised at various points, the formation of a new primordium has been observed in a space of about half the normal size (Wardlaw, 1949*c*). In apices where primordium P_3 and position I_1 were punctured, for example, an induced primordium has arisen in the space between, i.e. in approximately half the normal space as measured by its tangential component.

At the suggestion of Mr. R. Snow, the writer has limited the space available for the formation of a primordium by making two parallel radial cuts close together so as to enclose the I_1 or the I_2 position, with the results illustrated in Text-figs. 16–20. In a number of specimens, where the cuts were so close together as almost to coalesce, primordia were not formed. In other apices,

where the cuts were also rather close together (Text-fig. 16), no primordium was formed between the cuts but primordia labelled $I_{1'}$ and $I_{2'}$ arose beyond the innermost extremities of the cuts while I_3 and I_4 were formed in their normal positions. This result was frequently obtained. In other apices a primordium originated between the cuts, thus affording evidence of primordium formation in about one-third of the normal 'available' space as measured

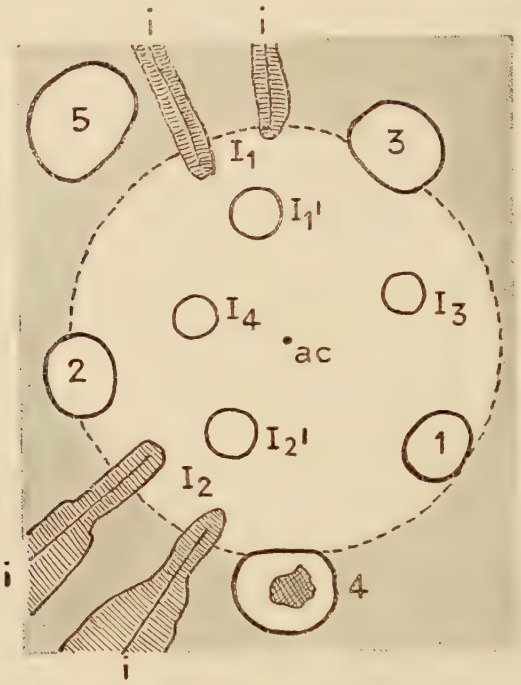


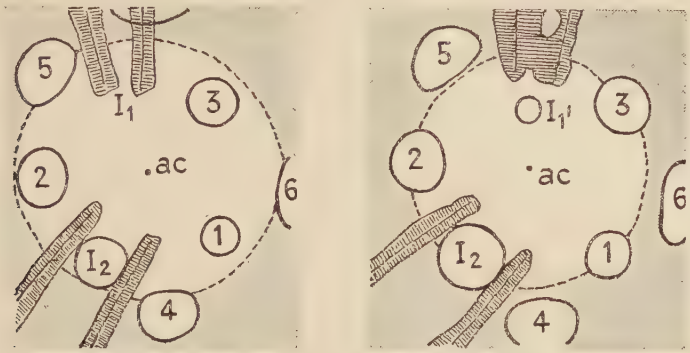
FIG. 16. An apex in which parallel incisions (*i*) have been made so as to enclose the I_1 and I_2 positions: new primordia which in due course appeared are shown. (×27.)

by the tangential component (Text-figs. 17–20). The illustrations also show that the isolated primordia were characterized by rapid relative growth, as already described (Wardlaw, 1949, 1949*c*). In these experimental materials the knife cuts gape slightly, the exposed tissue becomes brown and corky, while within, in proximity to the wound, there is always some development of parenchyma. Thus, where two parallel cuts are close together, the area of meristematic cells from which a primordium could be formed is small compared with a normal interfoliar area.

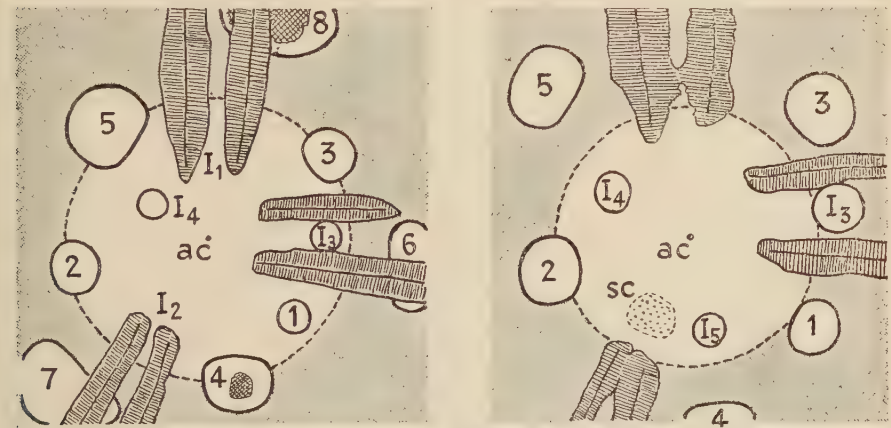
(*f*) *Anatomical observations*

Selected experimental apices were fixed, embedded in wax, and cut in serial transverse sections. Relevant anatomical observations are illustrated in Pls. XV and XVI. The general result of this aspect of the work has been to confirm the external observations illustrated diagrammatically in the text-figures.

Pl. XV, Fig. 1, shows the positional relationships of an induced leaf primordium (p) and a displaced leaf primordium (I_4) to an I_1 position which had been punctured; Fig. 2 of this series, taken lower down in the shoot, shows the



FIGS. 17 and 18. Experimental treatment as in Fig. 16: the same apex is shown on two successive weeks. Fig. 17: A leaf primordium has been formed in the narrow panel of tissue in the I_2 position, but no primordium has yet appeared at or near the I_1 position. Fig. 18: primordium I_2 has shown rapid relative growth and a second primordium, labelled I_1', has now appeared; *ac*, apical cell. (×20.)



FIGS. 19 and 20. Experimental treatment as in Figs. 16 and 17, but incisions have also been made so as to enclose the I_3 position: the same apex is shown on two successive weeks. A leaf primordium has been formed in the I_3 position and has shown rapid relative growth, Fig. 20. Primordia I_4 and I_5 are in their normal positions. *sc*, accidental superficial scar; *ac*, apical cell. (×20.)

vascular strands and leaf gaps relating to these two primordia. Observations such as these show clearly that the distribution of vascular tissue in the shoot is determined by the shoot apex and the lateral members to which it gives rise. In the present instance, the positions of the leaf-gaps shown in Fig. 2 are determined by two primordia, one of which (p) would not be present in the normal development, while the other (I_4) is displaced from its normal position. Again, in Pl. XVI, Figs. 10–12, the leaf primordium which has arisen

in the I_3 position between two close parallel incisions has an anomalous leaf-trace, i.e. it conforms to the modified outer contour of the shoot and to the space available for development. The highly varied and irregular vascular patterns in apices in which both leaf primordia and buds have been induced lend further support to the view that the initial differentiation of vascular tissue, i.e. its actual inception, is due to the basipetal movement of a substance (or substances) from the growing points of shoots, shoot buds, and leaf primordia (Pl. XV, Figs. 3–6).

The writer has previously shown that a lateral bud in *Dryopteris* and other leptosporangiate ferns typically arises in a position somewhat above the axil of a leaf. This is shown in Text-figs. 10–15 and is confirmed by the anatomical details illustrated in Pl. XVI, Figs. 7–9, 13.

IV. Discussion

Although the investigations described here have brought to light phenomena for which no adequate explanation can yet be offered, the data obtained support the view that growth centres and their physiological fields do exist at the shoot apex in ferns and that the phyllotactic sequence and, more generally, the organization of the leafy shoot and its regulated or harmonious development, can be referred to them. The data, in fact, afford the basis for a unifying conception of primary morphogenetic processes in vascular plants. It may be assumed that the physiological fields associated with the apical group of cells (the apical cell and its adjacent segments) and with the young leaf primordia have many properties, but, from the standpoint of organ formation and position, the inhibitional properties of fields are among the more important. In this connexion the finding of M. and R. Snow that the space on the apical meristem in which the next leaf primordium will arise must be of a certain minimal area receives support, in that this space must be sufficiently large so as not to be completely eclipsed by the inhibitive fields of the adjacent primordia. But the space needed for the site of the primordium may be quite small—a fact demonstrated in the experimental studies described here.

The inhibitional effect of the apical group of cells apparently reaches well down the sides of the apical cone: hence it is that new leaf primordia originate close to the basiscopic margin of the apical meristem. In the ferns, leaf and bud primordia are histologically identical: each originates from a small group of the superficial prism-shaped cells that constitute the apical meristem. At a certain distance below the apical cell, then, any group of these superficial meristematic cells is potentially capable of giving rise to a lateral member. But, in fact, lateral members arise at wide intervals in a regular sequence. According to the present investigations, the position occupied by a new leaf primordium is determined by the fields of the two older primordia adjacent to it and not by all the primordia of the top cycle. This again is confirmatory of the findings of M. and R. Snow. Between the widely spaced leaf primordia lie areas of the apical meristem. Now, why are no lateral members formed in

these areas? These interfoliar areas include bud positions, i.e. bud rudiments, but, in the normal development of the shoot, bud formation is completely inhibited. The bud rudiments remain inactive, as far as organogenesis is concerned, and are left behind when the shoot apex grows on. If, however, all the leaf primordia of the top cycle were punctured and the intensity of their fields thereby diminished, the expectation would be that lateral members would arise in interfoliar, i.e. in bud, positions. That this does happen has been abundantly demonstrated during the present investigations. Where the shoot apex is still intact, these induced members are seen to have the symmetry and configuration of leaves. Leaf formation can thus be induced in what is normally a bud position.

The orderly development of the leafy shoot is referable to the continued activity of the apical cell and to the regulated inception of growth centres in the apical meristem. While the leaf primordia of the top cycle preclude the formation of interfoliar members in that cycle, the new leaf primordia which are formed on the apical meristem, i.e. constituting the next cycle above, also play a part in the continued inhibition of the interfoliar members. Here we have evidence of those reciprocal relationships by which the activity of the shoot apex is characterized (Wardlaw, 1948): for whereas the positions and rates of growth of new leaf primordia are determined by the older leaf primordia below—an acropetal effect—the inhibition of interfoliar members is partly due to primordia above—a basipetal effect.

That the fern apex is totipotent in respect of organ formation seems evident from the accumulated data of these studies. In experiments (section III (*d*)) where both shoot and leaf apices were punctured, leaf primordia arose in some of the presumptive leaf positions and buds in others. It thus appears that very little is needed to tip the balance from leaf formation to bud formation. If it be assumed, as has been done here, that there are no fundamental metabolic differences between leaf and bud primordia (i.e. that no special 'leaf-' or 'bud-forming' substances are involved), then the symmetry and configuration of an organ must be determined by the position in which it is formed and by the physical and physiological factors incident in that position. If, on the other hand, it is assumed that specific 'organ-forming' substances are involved, then it becomes necessary to explain how these substances come to be located, at critical concentrations, in particular positions. In the experimental materials under consideration there would be the added difficulty of explaining how, in some instances, a 'bud-forming' substance has become localized in what is normally a leaf site and vice versa. Any general hypothesis of the kind that leaves inhibit leaves and buds inhibit buds—though it offers attractive possibilities—is confronted with the same difficulties. Hence, as it seems to the writer, it is to the mechanics and genic control of growth in the plastic growing region that we must look for an explanation of the characteristic form and structure of the shoot and its lateral members. In the ferns, leaf primordia are formed on the sides of the apical cone in sites which are characterized by marked differences in the rates of growth on the adaxial and abaxial sides:

buds, on the other hand, arise on older regions of the shoot where this marked asymmetry of position and of growth has disappeared. Buds are only induced on the apical meristem after the sites have been isolated from the apical cell or when the latter has been punctured. In both instances the asymmetry of position which characterizes leaf formation has to some extent been removed. No doubt this tentative account of factors which may account for the differences between leaves and buds is an over-simplification, but the data of section III (*d*) show that these differences may not be so great as they appear to be.

According to the growth-centre hypothesis, a new centre can only originate on the meristem outside the effective field of an existing centre. In apices which have been subjected to experimental treatment, the possibility of two growth centres originating simultaneously presents itself and, as the text-figures show, two primordia may arise in close proximity: the development of such primordia and, in particular, their rates of growth merit further consideration.

In the development of the fern plant from the young sporophyte to large adult size, the apical meristem undergoes a progressive increase in size, the apex of the adult being, in general terms, a magnified image of the small apex of the young sporophyte (Wardlaw, 1948). But in an enlarging conical structure such as the apex, certain geometrical relationships are constantly changing, e.g. the actual distance between adjacent leaf primordia increases. Although at present we have no knowledge of the extent or shape of the physiological fields surrounding the leaf primordia in a young sporophyte as compared with those at the apex of an adult plant, nevertheless the probable importance of relevant geometrical aspects is evident. In due course we may therefore expect to see a renewal of interest in the observations and findings of investigators such as Van Iterson (1907).

Interesting as are the results that have been obtained by the methods used here, such an approach has definite limitations. Further investigations of this kind are not precluded, but the next phase of this research clearly lies in the field of the physiologist. It would, for example, be of great interest to know to what substance, or factors of growth, the inhibitional properties of the fields postulated here are due and if there is, in fact, a differential distribution of a growth-regulating substance in the apical meristem. Then, again, if the postulated fields do exist, it seems likely that they will have a three-dimensional aspect, and it may be that the component at right angles to the superficial meristem is effective in bringing about the inception of the vascular tissue which is to be seen immediately below the apical meristem and the organs that originate there. These and related problems would appear to offer a fruitful field for morphologists and physiologists working in conjunction.

On the basis of earlier work on *Dryopteris* and other ferns it was concluded that the position of a bud lies somewhat *above* the leaf axil, not *in* the leaf axil as in flowering plants (Wardlaw, 1943, 1943*a*). The observations on bud

formation which have been described and illustrated here fully support the earlier conclusion. From these observations it may be inferred that the inhibitive field of a leaf primordium is inextensive in the acropetal direction, its main effects being lateral and basipetal. If this is so, the inhibition of buds above the axils of the top cycle leaf primordia, i.e. P_1–P_5, will be due to the apical cell group, whereas, as many observations show, the inhibition of buds in interfoliar positions, i.e. lateral to P_1–P_5, is due to these leaf primordia.

The effective physiological field of the apical cell group may be envisaged as extending down the sides of the apical cone as far as the axils of P_1–P_5, the adaxial sides of which show evidence of inhibited growth. An extreme case of this inhibition is seen in *Matteuccia struthiopteris*, where deep axillary cavaties or pockets are formed. It is tempting at this stage to discuss in some detail the shapes of the physiological fields at the apical meristem. Nevertheless, because of the complex nexus of factors involved in the growth of the shoot apex and the many physiological aspects as yet unexplored, it seems advisable to leave the matter as a general statement until further data have been obtained. In zoological literature the field concept has now been elaborated and discussed in very considerable detail by investigators such as Huxley (1935), Weiss (1939), Needham (1942), and others. By comparson, botanists have lagged behind, though one of the first contributions to this subject was made by Gurwitsch (1922) using examples drawn from botanical sources.

V. Summary

1. Further evidence has been obtained in support of the view that the extreme apex of the fern shoot and each young leaf primordium constitutes a growth centre with a surrounding physiological field and that these determine the inception of leaf primordia, the regular sequence in which they appear, and the harmonious development of the leafy shoot.

2. By destroying the leaf primordia of the top cycle, interfoliar leaf primordia can be induced; these occupy what in the normal development would be bud positions (bud formation being normally inhibited). Although the inhibition of these interfoliar primordia is due to the top cycle of primordia, the next cycle of primordia to be formed on the meristem also play a part in maintaining the inhibition.

3. In specimens in which the shoot apex and the apices of the top cycle of leaf primordia were punctured, interfoliar primordia were induced. Of the lateral members which in due course appeared in the I_1, I_2, and I_3 positions, some proved to be leaf primordia and some buds. The apical meristem in ferns is thus seen to be totipotent in respect of organ formation. Small differences in the incidence of physical factors and factors of growth apparently determine whether a leaf or a bud will be formed.

4. A leaf primordium may arise in a half or even one-third of the normal 'available space' between primordia. If a leaf primordium, together with its physiological field, is taken as a morphogenetic unit, the view of M. and

R. Snow that the next primordium to be formed will arise in the 'next available space' is held to be valid.

The writer has pleasure in acknowledging the assistance received from Mr. E. Ashby in microscopic preparations and photographic illustrations.

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EXPLANATION OF PLATES

Illustrating Professor C. W. Wardlaw's paper on Induction of Leaves and Buds in *Dryopteris aristata* Druce.

(All figures are from untouched photographs)

PLATE XV

Fig. 1. An experimental apex in transverse section. The position of I_1 was punctured. An induced leaf primordium (*p*), axillary to P_8, has appeared laterally; a new primordium I_4 has also been formed in close proximity to the wound at I_1; *vt*, incipient vascular tissue. (×50.)

Fig. 2. As in Fig. 1, but lower down in the shoot, showing the leaf-traces and leaf-gaps of *p* and I_4. (×50.)

Figs. 3 and 4. The apex illustrated in Text-fig. 13 cut in transverse section, showing the complex and greatly modified pattern of the vascular system (*vt*) in Fig. 3, and the approach to a more normal distribution of vascular tissue lower down, Fig. 4. (×15.)

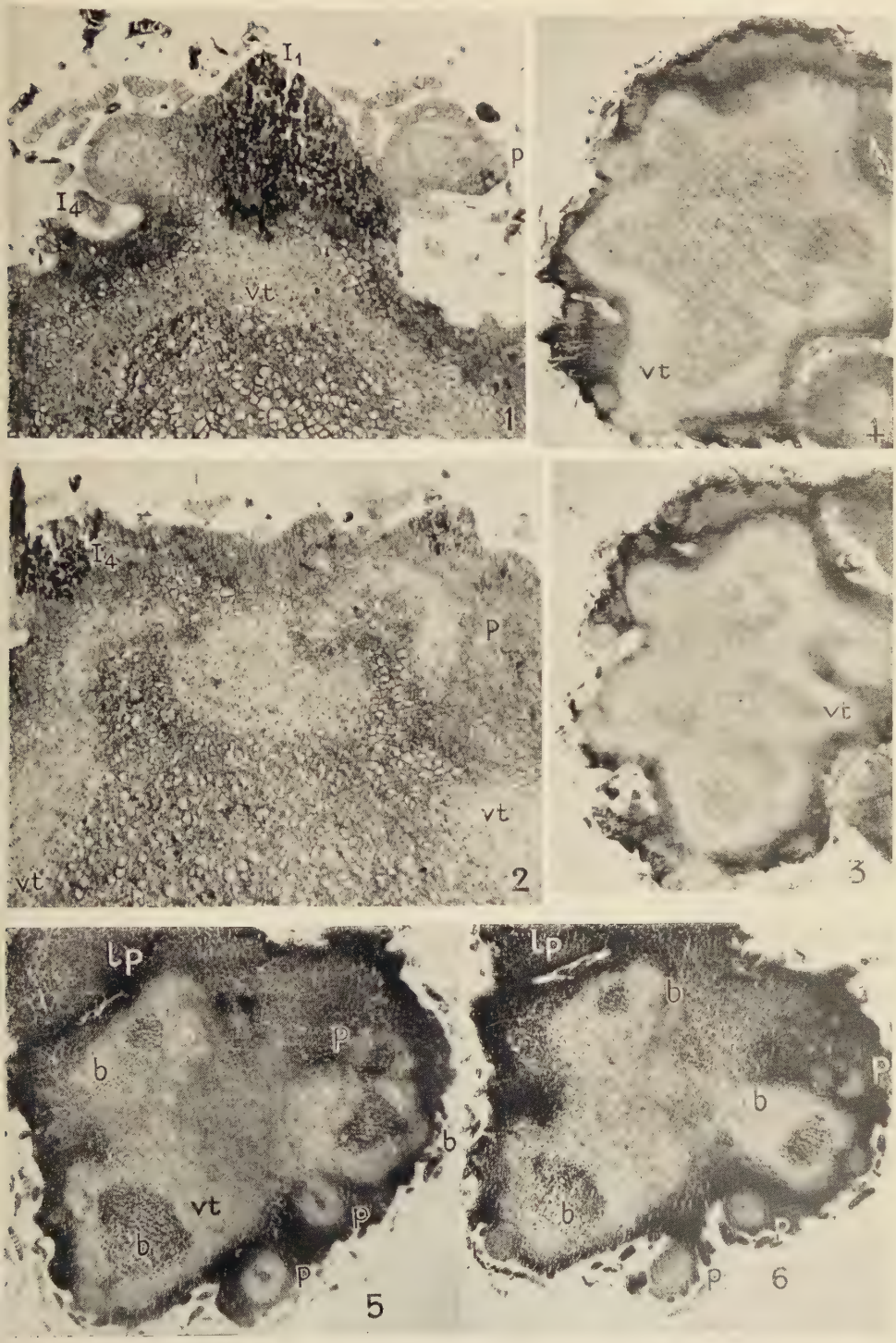
Figs. 5 and 6. Transverse sections of the experimental apex illustrated in Text-fig. 12. The solenostelic vascular tissue (*vt*) of three induced buds (*b*) and the horseshoe-shaped traces of induced leaf primordia (*p*) can be seen; *lp*, leaf primordium, with its axillary bud (*b*). Fig. 6 is closer to the apex than Fig. 5. (×24.)

PLATE XVI

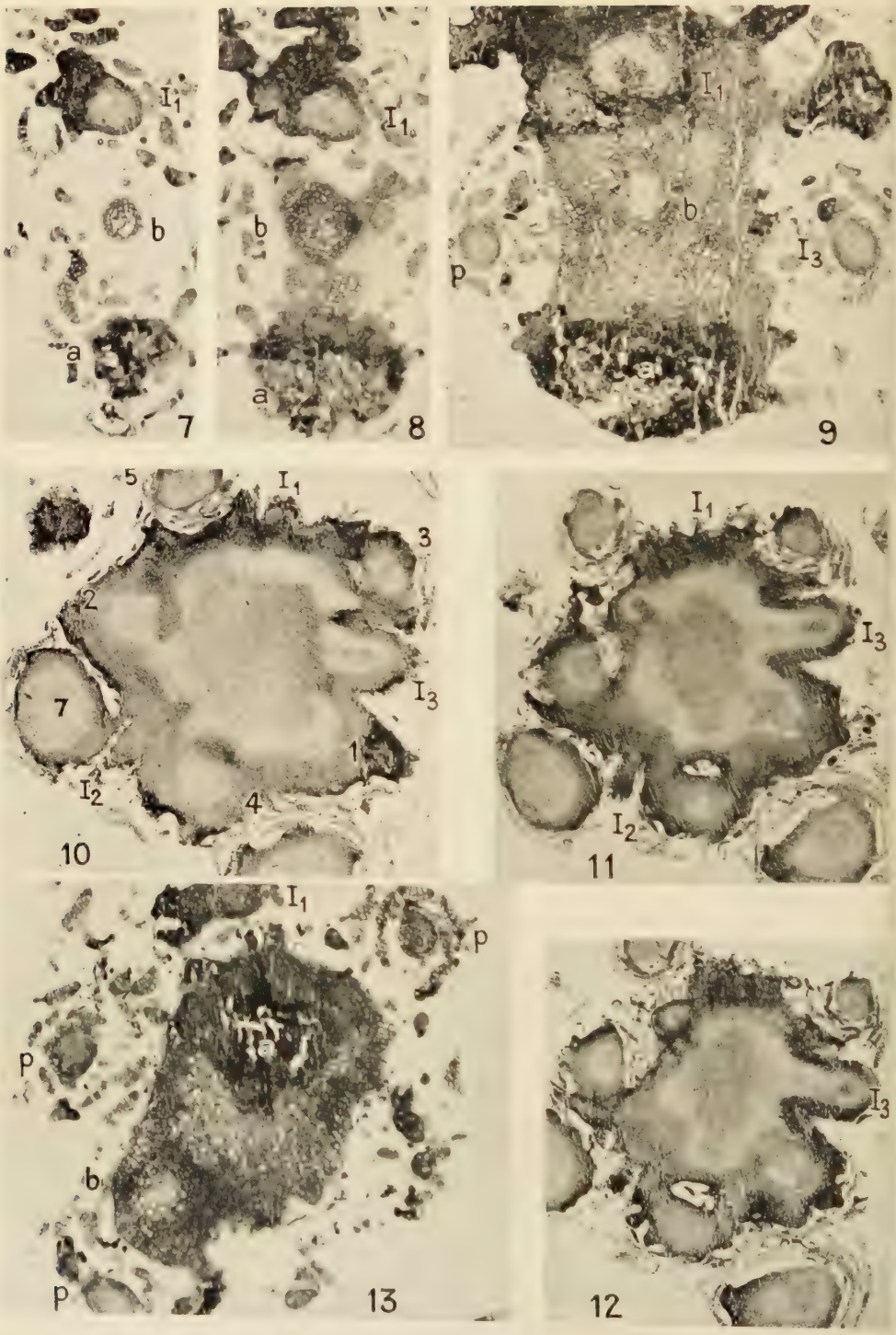
Figs. 7, 8, 9. The apex illustrated in Text-fig. 15 in transverse section at three levels. The shoot apex (*a*) was punctured; a leaf primordium was formed at I_1, and a bud (*b*) subsequently arose above its axil; the leaf primordium at I_3 is seen in Fig. 9, and also an induced primordium (*p*). (×40.)

Figs. 10, 11, 12. The apex illustrated in Text-fig. 20, in transverse section, at three levels. No leaf primordia have arisen between the parallel radial incisions at I_1 and I_2, but one did arise between the incisions at I_3; the somewhat modified leaf-trace can be seen; *1*, *2*, *3*, &c., the existing leaf primordia in order of age. (×15.)

Fig. 13. Transverse section of the apex illustrated in Text-fig. 11. The apical cell (*a*) was punctured; a bud (*b*) has been formed above the axil of an induced leaf primordium (*p*); other induced leaf primordia (*p*) and the leaf primordium which arose at I_1 can be seen. (×35.)



C. W. WARDLAW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I1
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11
I1
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b
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12

Two New Algae from Indian Soils[1]

BY

A. K. MITRA

(*Department of Botany, Queen Mary College, London, and University of Allahabad, India*)

With forty-five Figures in the Text

THE algae described in this paper appeared in cultures of desiccated soils collected from cultivated and uncultivated land near Allahabad, India. The new cultures were prepared with De's (1939, p. 124) modification of Benecke's solution. The algae involved were reared in unialgal cultures, mainly obtained by streaking on agar, followed by growth in De's solution with a little soil extract or on solid media prepared by adding 1·5 per cent. agar to the preceding.

1. *Chloranomala palmelloides* gen. et sp. nov. (Figs. 1–24)

The palmelloid colonies of this alga appear as a thin, light green, easily breakable film on the surface of the culture solution or on the sides of the vessel. The film consists of a hyaline mucilaginous matrix containing globular cells in all stages of division. The individual cell is surrounded by a special hyaline mucilage layer, which varies in thickness according to its degree of hydration and is easily seen after staining with ruthenium red (Fig. 1). The cells possess a light green parietal chloroplast, with lobed edges, not occupying the entire contour of the cell and containing a pyrenoid with a starch-sheath. Staining with iodine, ruthenium red, or Noland's reagent fails to reveal the presence of pseudo-cilia.

Before division the side of the cell away from the pyrenoid broadens so that the cytoplasm harbouring the nucleus becomes more obvious (Fig. 2). The pyrenoid divides by constriction. The daughter-cells are usually of equal size (Figs. 3, 4), although sometimes very unequal (Fig. 5). The first division is soon followed by a second in a plane at right angles to the first, the two daughter-cells of the first division dividing simultaneously or successively (Fig. 6). The four resulting cells slowly separate as mucilage is secreted and gradually acquire a rounded shape (Figs. 6–10).

When transferred to water the mucilage swells and the cells become widely separated. The protoplasts acquire flagella and, in 20–30 minutes, wriggle out as naked swarmers. Meanwhile a marked change takes place in the appearance of the cell-contents. The chloroplast becomes paler and obscured by an accumulation of large numbers of small oil-globules, with a bluish-green glint and situated chiefly in the neighbourhood of the chloroplast. The pyrenoid becomes difficult to recognize and, when visible, the starch-sheath

[1] Part of a thesis presented for the Ph.D. degree of the University of London.

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appears much thinner or may even be absent (Figs. 9,10, 13). During swarmer-formation most, if not all, of the starch seems to be converted into oil and treatment with iodine affords very little blue coloration.

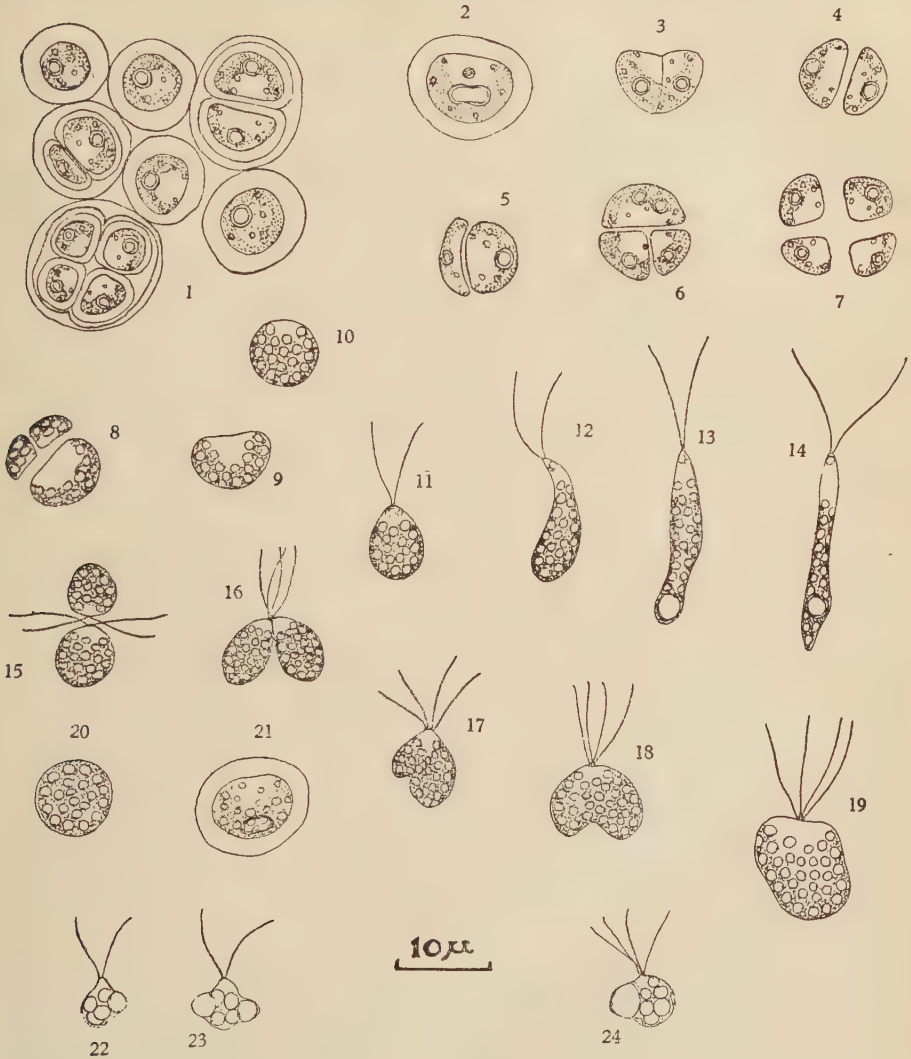
The swarmers (Figs. 11–14) possess two equal flagella, attached to the hyaline anterior end and about as long as the body. The swarmers are highly metabolic, at times being globular or ovoid and then measuring 3–5 μ in diameter, while at other times they may lengthen to about 20 μ. The thin cup-shaped chloroplast is obscured by the numerous oil-globules. The pyrenoid is rarely discernible, and is seen especially near the posterior end of the much elongated individuals (Figs. 13, 14), but its position is variable, owing to the metaboly of the cell. Two contractile vacuoles, pulsating alternately, lie in the clear cytoplasm at the anterior end. There is no eye-spot and the swarmers are almost indifferent to the direction of the incident light.

Except for a small anterior region, the swarmer is filled with the above-mentioned globules, which give all the tests for fatty oil. When the cells are crushed, the globules coalesce to form large drops which, like the globules within the swarmer, stain bright red with Scharlach red. With osmic acid they gradually blacken. When treated with dilute iodine the swarmers contract and the unstained globules coalesce into a few larger ones, some of which protrude from the cells (Figs. 22–4). The globules are completely dissolved by benzol and subsequent treatment with Scharlach red, after removal of the benzol, affords no coloration. The various tests have been repeatedly performed and suggest that the globules are fat.

The swarmers continue to move for 5–8 hours and finally come to rest on the surface or at the bottom of the liquid. They assume a spherical shape and soon secrete a mucilaginous envelope. After a considerable length of time the oil-drops diminish in number, and the chloroplast and pyrenoid again become prominent.

The swarmers may also behave as facultative gametes which fuse during movement. They aggregate in clumps, comprising 3–50 swarmers, all of which retain their motility. Although there is frequent conjugation between two equal gametes (Figs. 15, 16), a larger gamete is usually seen to be followed by two or three smaller ones, with one of which it fuses (Fig. 17). The gametes come together by their anterior ends, becoming entangled by their flagella (Fig. 15), and then coming to lie side by side (Fig. 16). Fusion commences at the anterior and progresses towards the posterior end (Figs. 17, 18). The process of pairing takes about 10–15 minutes, but fusion is completed in about a minute. The naked quadriflagellate zygote (Fig. 19) continues to move for several hours, ultimately, like the swarmers, rounding off and secreting a mucilage envelope (Figs. 20, 21). After a time division into four daughter-cells takes place. Although this organism has been cultivated in unialgal cultures on a variety of solid and liquid media for a considerable time, no resting-stages have ever been observed. On drying agar the envelope becomes firmer and most of the cells die. When placed in water the contents of the surviving cells emerge through a gelatinized part of the envelope.

The nature of the chloroplast, the production of starch, and the presence of two equal anterior flagella in the swarmers indicate that this alga should be classed among the Chlorophyceae. The palmelloid colonies, in fact, suggest



FIGS. 1–24. *Chloranomala palmelloides* gen. et sp. nov. Fig. 1. Cells embedded in mucilage. Figs. 2–7. Stages in cell-division. Figs. 8–10. Appearance of oil-globules in incipient swarmers. Figs. 11–14. Swarmers. Figs. 15–18. Stages in sexual fusion. Fig. 19. Zygote. Figs. 20–1. Vegetative cells derived from zygotes showing secretion of mucilage. Figs. 22–4. Swarmers and zygote stained with iodine, showing coalescence of oil-globules.

a reference to the Palmellaceae, and the cell-structure would be in agreement with this. The copious formation of oil-globules, which precedes the liberation of swarmers and characterizes the motile stages, is a unique feature that

renders it impossible to refer this alga to any known genus of Green Algae. The possibility of a parasite being involved was examined critically. Unialgal cultures were prepared several times by streaking on agar. Single swarmers were isolated and observed throughout their development, but no evidence of the presence of a parasite was ever obtained.

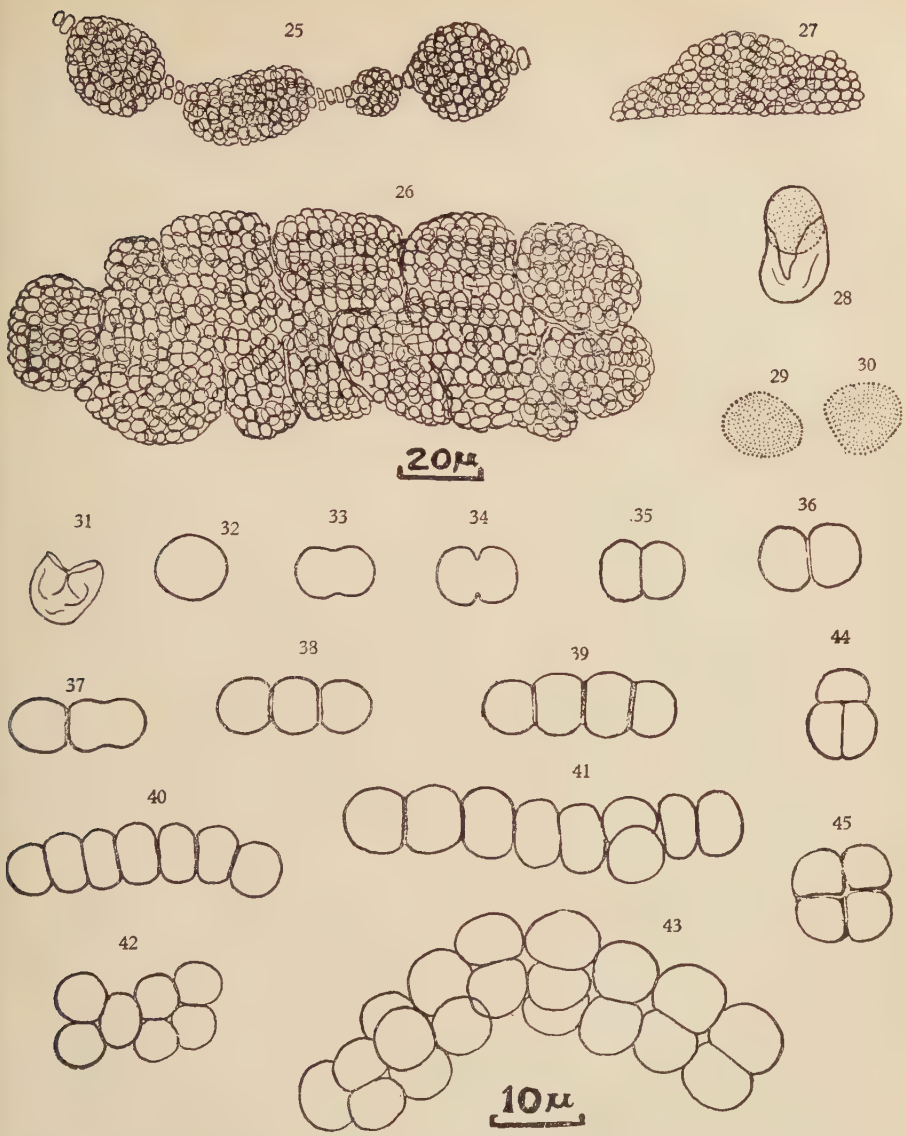
The method of sexual reproduction resembles to a certain extent that described for *Tetraspora lubrica* by Geitler (1931, p. 177), but there are important differences in the structure of the gametes and in their metaboly and physiological behaviour. Moreover, as no evidence of the presence of pseudocilia has been obtained, an affinity with Tetrasporaceae is doubtful. The alga may, therefore, provisionally be referred to the Palmellaceae.

2. *Chlorogloea Fritschii* n. sp. (Figs. 25–45)

This alga formed a deep blue-green amorphous crust, about 500 μ thick, on the sides of the culture vessel, as well as on soil, and a thinner, though more expanded, stratum on the surface of the liquid. These growths consist of large, loosely and irregularly superposed, packets which are visible to the naked eye and consist of rounded chroococcoid cells (Fig. 26) of variable size. No mucilage-envelope is recognizable around the individual packets, even after staining with ruthenium red or mounting in indian ink, although the cells cohere firmly. The cells themselves are also without any evident enveloping mucilage, although the cell-wall stains with ruthenium red, methylene blue, or cotton blue. In the young condition the cells are arranged in longitudinal and transverse rows (Fig. 43), but this arrangement is later obscured by displacement during division and enlargement. Although generally rounded, the cells may become polygonal by mutual pressure. In older cultures the membranes of the peripheral cells become pale brown and, after discharge of the contents, the empty walls always show a distinct brownish tinge. The cell contents are pale blue-green and contain a few large scattered granules.

Cell division takes place by gradual constriction along three planes at right angles to one another. As a general rule the cells enlarge and round off after division, but groups of four cells are often observed. Within the individual packets the cells, in optical section (Fig. 27), appear arranged in vertical and horizontal rows.

Any cell can produce a single endospore, liberated by rupture of the envelope (Fig. 28) which subsequently collapses and becomes folded (Fig. 31). The endospores are naked, devoid of a mucilage-envelope, and correspond in shape to that of the parent cell. They are usually globular, but sometimes a little flattened on one side (Figs. 29, 30). The contents are pale blue-green and finely granular. Sometimes the endospores undergo constriction before liberation and in rare instances may even exhibit division into two. These are probably examples of premature germination, since the cells remain joined after liberation and do not constitute separate endospores. Sometimes an ordinary cell of a packet divides into a number of small ones, but each such cell has an envelope of its own and can liberate an endospore.



FIGS. 25–43. *Chlorogloea Fritschii* n. sp. Fig. 25. Part of a growth from a liquid culture containing ammonium salts. Fig. 26. Usual growth, showing packets of cells. Fig. 27. Section of a packet. Fig. 28. Liberation of endospore. Figs. 29–30. Naked endospores. Fig. 31. Empty membrane after liberation of endospore. Figs. 32–40. Stages in the germination of endospores, showing formation of a uniseriate filament. Figs. 41–3. Formation of filaments with two or three rows of cells. Figs. 44–5. Division of detached cells.

The naked endospores subsequently always assume a globular shape and secrete a delicate envelope. After slight elongation they divide into two by constriction (Figs. 33–6). Further divisions in the same plane (Fig. 37) give rise to a short, straight or curved, uniseriate filament of 3–12 cells which is

clearly constricted at the septa (Figs. 38–40) and without any mucilage-envelope. The outer surface of the end-cells is broadly rounded, and these cells are sometimes larger or smaller than the others. Subsequently certain cells of the filament begin to divide along a plane at right angles to that of the earlier ones (Fig. 41), and by degrees other cells follow suit. These divisions lead to the formation of a biseriate filament (Fig. 42), but this stage is transitory, since divisions soon take place along three planes at right angles, so that the filamentous character becomes obscured (Fig. 43). Since every cell of the primary filament divides in the same way and similar divisions take place in the daughter-cells, the cells of the resulting packets are arranged in vertical and transverse rows. Endospores often germinate near their place of liberation and may form packets overlying that from which the spore was produced.

Another method of reproduction consists in the separation of single cells or of groups of two or three cells from the packets. These develop into short filaments in the same way as the endospores, but division in all three planes usually sets in early.

In liquid cultures, and especially in media containing ammonium nitrate, only some of the cells of the primary filaments continue to divide and form packets, while others, with or without division, begin to degenerate. This gives the resulting growth a jointed appearance (Fig. 25), with irregular packets of blue-green cells strung together by strips in which the degenerating cells appear yellowish. This rarely happens in agar cultures.

The mature growths of this alga resemble a Microcystis, except for the orderly arrangement of the cells in rows and the frequent grouping of the packets in linear series. This latter feature suggests a reference to the Entophysalidaceae (Fritsch, 1945, p. 818). There are some resemblances to *Chlorogloea microcystoides* Geitler (1925, p. 357; 1932, p. 310), the only freshwater species so far recorded, in which, however, the early development is unknown. The Allahabad alga may be provisionally referred to Chlorogloea, pending an investigation of the early stages of *C. microcystoides*, though the differences may prove to be great enough to warrant the establishment of a separate genus.

The general shape of the growths and the disposition of the cells in rows constitute the chief resemblances to *C. microcystoides*, but the habitat is different and there is no common mucilage-envelope around the packets; the cells are also larger and more loosely disposed. When viewed in optical section the rows of cells afford no evidence of the branching seen in Geitler's species. The most important difference, however, lies in the method of reproduction. In *C. microcystoides* a number of endospores are formed within enlarged cells, while in *C. Fritschii* the endospores are produced singly within ordinary cells. The formation of a primary filament has not been recorded among the Entophysalidaceae, although it occurs in diverse Pleurocapsales, such as *Pleurocapsa minor* Geitler (1925, p. 343). Here, however, the terminal cell of the filament behaves as an apical cell and shows oblique division.

Chlorogloea Fritschii also shows some resemblance to *Chroococcopsis*

fluminensis Fritsch (1929, p. 183), which Geitler (1932, p. 323) would include in *Pleurocapsa minor* owing to occasional production of upright rows of cells. *Chroococcopsis fluminensis* forms a similar deep blue-green stratum consisting of loose aggregates of chroococcoid cells giving rise to single endospores, whose germination is unknown.

If *Chlorogloea Fritschii* is rightly referred to the Entophysalidaceae, it affords a link between this family and the Pleurocapsaceae. Fritsch (1942, p. 138; 1945, p. 858) doubts the derivation of Pleurocapsales from the Entophysalidaceae, advocated by Geitler (1932, p. 94 and p. 293), since there has hitherto been no evidence of the occurrence of a juvenile filament in the Entophysalidaceae. The presence of such a stage in *C. Fritschii*, however, appears to lend support to such a derivation.

Diagnosis of the New Forms

Chloranomala palmelloides gen. et sp. nov.

Cells globular or hemispherical after division, 4–8 μ in diameter, embedded in soft mucilage to form a thin film; chloroplast parietal, cup-shaped with lobed edges, light green, the pyrenoid usually opposite the opening of the chloroplast. Multiplication by division of cells along two planes at right angles to one another, the daughter-cells provided with individual mucilage-envelopes. Swarmers with two equal flagella strongly metabolic, ovoid to elongate, 3–5 μ in diameter and up to 20 μ long, the parietal chloroplast with a basal pyrenoid obscured by numerous oil-globules, contractile vacuoles two, anterior; no eye-spot. Swarmers can behave as gametes, showing iso- or anisogamous fusion after clump-formation; zygote 4-flagellate, motile, like the non-conjugating swarmers rounding off after some time and dividing to form a new palmelloid colony.

Hab. In cultures of desiccated soils from rice-fields and gardens, as well as from usar and red soils, India.

Chlorogloea Fritschii n. sp.

Stratum a deep blue-green crust of indefinite size, composed of rounded or irregular cell-packets. Cells arranged in vertical and horizontal rows, rounded or angular, without evident mucilage-envelopes, with pale blue-green granular contents, diameter 4–12 μ (usually 6–8 μ). Reproduction by (1) naked spherical endospores, 4–9 μ broad, formed singly within the cells and liberated by rupture of the membrane, and (2) separation of single cells or groups of two or three cells. Endospores give rise to a short prostrate uniseriate filament of 3–12 cells which divide in three directions to produce the packets.

Hab. In cultures of cultivated and garden soils from Allahabad, Northern India.

Summary

A new monotypic genus *Chloranomala palmelloides*, provisionally referred to the Palmellaceae, is described. The highly metabolic motile cells contain

large quantities of oil globules and either germinate without fusion or behave as gametes.

A new species of *Chlorogloea* (*C. Fritschii*), whose endospores germinate to produce an at first uniseriate filament, is described.

The author is indebted to Professor F. E. Fritsch, F.R.S., for advice and guidance during this investigation.

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Growth Analysis of the Sugar-cane Crop in North Bihar (India)

I. Seasonal Variation in Growth and Yield in Unmanured Plots

BY

R. D. ASANA

(*Indian Agricultural Research Institute, New Delhi*)

With five Figures in the Text

Introduction

IN North Bihar in north-east India is located the second largest white-sugar belt in India. This tract is situated in the sub-tropical Indo-gangetic plain and is perhaps the only one of its kind in the world where sugar-cane as a commercial crop is grown without irrigation.

The climate of North Bihar has a fairly cold winter with sometimes a little rain at intervals. Frost is very rare. From March to June the weather is hot and the maximum temperature occasionally rises above 100° F. There are often storms towards the end of the hot weather and frequently useful rain. The monsoon usually breaks by the middle of June and from then to the middle of September an average rainfall of 45 in. (110 cm.). Vegetative growth of sugar-cane practically ceases by the middle of October.

The soil varies from light sandy loam to medium silty loam. There is a high proportion of lime. The soil is interspersed with beds of sand through which water seeps sideways underground from the rivers. It is this combination of a comparatively mild climate with the fertile moisture-holding and supplying soil that makes cane-growing without irrigation possible.

Sugar-cane is grown as an annual crop in North Bihar, and the average yield is perhaps the lowest in India, approximating to about 15 tons per acre. The Coimbatore canes are grown over almost the whole area. The problem of increasing tonnage per acre is very pressing. Manuring with nitrogenous and phosphatic fertilizers has, however, been found to have an inconsistent effect. It was therefore considered worth while to undertake a physiological analysis of growth under field conditions on the lines formulated originally by Gregory (1917) and Briggs, Kidd, and West (1920). Similar studies undertaken in the case of cotton by Crowther (1934, 1941) in the Sudan and Heath (1937) in East Africa and in the case of beetroot and cereals by Watson (1938, 1947) in England have yielded valuable results. Observations reported in this paper were made at Pusa, where conditions are representative. The effects of nitrogen, phosphorus, and irrigation on growth and yield were investigated during

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four seasons. Observations on unmanured plots alone are reported here. The variety used, Co 313, is very widely cultivated in this region.

A brief reference to the usual agronomic practice may not be out of place. The second half of February appears to be the most suitable time for large-scale planting, as the temperature then is not too low and soil moisture is just enough for germination to commence. With the progressive delay in the date of planting the soil moisture tends to diminish with rising temperature and decreasing relative humidity. Cane sets are planted end to end in a furrow opened by a ridge-plough. The furrow is then closed by a 4-tine Bihar cultivator and the field is then levelled off by beaming. Immediately after the monsoon has set and before the cane has grown too tall to get the bullocks through it without damage the crop is earthed up by driving a ridge-plough through the rows. The ridge-plough throws the earth up into ridges along the rows of the cane and leaves furrows between the rows. A 3-year rotation is the usual practice. In the preceding summer the land is kept either fallow or put under early paddy, maize, or a green manuring crop. At the time of preparatory tillage in early December, farmyard manure at the rate of $3\frac{1}{2}$ tons per acre is applied if the land has been under paddy or maize.

Experimental Details

The data under discussion relate to observations on control plots from field experiments with all combinations of three levels of nitrogen and phosphoric acid applied in the form of castor cake and double superphosphate respectively at the time of planting. The treatments were replicated three times and also randomized. Plot size was 60·5 ft. by 30 ft.—$\frac{1}{24}$ acre—and included ten rows 3 ft. apart. The central four rows ($\frac{1}{60}$ acre) were reserved for final yields and periodic observations on height, tillering, &c.; plant samples were collected from the other four rows, two on each side of the four central rows. The remaining two rows on each end of the plot served as border rows. The seed rate was constant throughout—64 healthy 3-bud sets per row (60·5 ft. long).

The first sample was collected about 8 weeks after planting when germination was complete and just before the appearance above ground of the first tiller. Samples were collected regularly at intervals of 4 weeks until the shoot ceased elongating. Thus about seven samples were collected during the growing period. Four clumps (aerial portion cut at ground level) were collected at random from each plot, one from each row. With three replications a complete sample per treatment consisted of twelve clumps. The size of the sample was fixed according to convenience and facilities available in the laboratory. Each row was divided into seven equal lengths, to each of which was assigned a number ranging from 1 to 7. From one of these numbered lengths the corresponding sample was collected, which consisted of a clump of average size (as judged by eye). Statistically randomized sampling had to be thus compromised because the sugar-cane crop is never very uniform, the time taken for germination (which was at most about 33 per cent.) extending over weeks during which the seedlings are also ravaged by pests.

In the laboratory the plant samples were separated into fully unfolded green leaves, sheaths, stems, and dry leaves. The partially unfolded green leaves were mixed with sheaths. After cutting the various parts into small bits of convenient size the whole sample was quickly weighed and a small sub-sample amounting to about 10 to 20 per cent. of the original weight was again weighed out and dried. Drying was effected in large wooden cabinets specially constructed for the purpose. There was no bottom to the cabinet, and the top could be slid open for letting out moisture-laden air. In the bottom was placed an electric heater over which rested a large tin sheet. The shelves were made from drawn metal. The sample trays were made from bamboo strippings. The upper shelves did not receive the same degree of heat as the lower ones and therefore all the samples were dried on the two lower shelves for the same length of time (at about 70° C.) before weighing. The dried samples were powdered in a hand-mill, sieved, and stored in sealed containers for chemical analysis later.

It was considered necessary to make some adjustment in the dry weights of the samples. Attacks by borers caused irregular damage to the shoots (dead hearts) and it was difficult to pick out from within an 8·5 ft. length a clump of average size. This difficulty was experienced particularly after the internodes elongated and cane formation started. The dry weight of the samples collected after this stage were, therefore, corrected on the basis of mean number of healthy canes per clump per plot (obtained from periodic shoot counts of the four central rows).

Shoots in the four central rows (reserved for yield) were periodically counted and the number of dead hearts was also recorded. Three shoots in each row were tagged at random and heights of twelve such shoots (from a fixed point at ground level to the transverse mark on the topmost fully expanded leaf) were also recorded at periodical intervals. Observations on the production and death of leaves were also recorded. If a shoot was affected by dead heart, it was substituted by another healthy shoot.

Methods of Growth Analysis

From the sampling data of dry weights estimates of net assimilation rate were obtained by methods used by Gregory (1926). The net assimilation rate was calculated on the basis of unit dry-weight of green leaf instead of unit area owing to the difficulty of estimating leaf-area in the field. From the dry-weight data and the nitrogen content the uptake of nitrogen was calculated as absolute rate of uptake. (Total nitrogen was estimated by the usual Kjeldahl method after reducing nitrate in the cold by the salicylic acid sulphuric acid mixture.) The importance of estimating the uptake of phosphorus and potassium was recognized, but lack of adequate laboratory facilities precluded their analysis.

The mean rates of production and mortality of tillers were calculated thus: if at times t_1 and t_2 the number of all shoots be N_1 and N_2 and the number of 'dead hearts' D_1 and D_2 respectively, then the rate of

production between interval t_1 to t_2 is $[(N_2-N_1)/N_1]\times 100$ and the rate of mortality is $[2(D_2-D_1)/(N_1-N_2-2D_1)]\times 100$.

Final Yields

In Table I are given the yields of millable cane along with other relevant particulars. It will be seen that there is an appreciable variation in yield from year to year. Difference in germination or cropping during the preceding summer does not correlate with variation in yield. Discussion of the causes of differences in yield would appear to be facilitated by comparing growth data of each experiment with those of Bhograsan (which gave the highest yield) taken as the standard. Only those data which bear directly on the points under consideration have been presented.

Table I

Field.	Soil type.	Preceding summer crop.	Mean yield (tons/acre).	Planting date.	Germination %.
North Pangarbi	Sandy loam	Greenmanure	12·96	14/2/42	30·0
Brickfield	Silt loam	Fallow	12·96	5/3/45	32·7
Nepali	Sandy loam	Fallow	15·80	24/2/43	32·0
Harpur Jhilli	Silt loam	Fallow	16·19	8/3/43	34·0
Chonia	Silt loam	Maize	19·02	3/3/44	26·0
Bhograsan	Sandy loam	Fallow	21·78	23/2/44	32·0

Developmental Observations and Sampling Data

Bhograsan and Nepali

The course of tillering is indicated (Fig. 1) as mean number of living shoots per clump plotted against time in number of days from planting. The tiller production in Nepali was identical with, or even slightly superior to, that in Bhograsan in the early stage, but lagged behind after about 13 weeks. The maximum difference between the two was reached by about 17 weeks. Subsequently the difference diminished, but the difference as regards the mean number of mature canes per clump at harvest was almost as wide as that between tiller numbers at 17 weeks. Evidently the tillers in Nepali that developed later did not mature into millable canes. It may also be noted that the mean percentage death-rate was consistently higher in Nepali up to about 17 weeks. This does not, however, account for the difference in tiller number by the end of the period because the mean rate of production was substantially higher in Bhograsan. It may be noted that the maximum on the tiller curve in Nepali was attained later and was also at a lower level than that in Bhograsan.

Maximum green-leaf weight was attained in both by 29 weeks. Difference in leaf weight between the two was apparent by 13 weeks; it widened gradually up to about 25 weeks and then diminished. Since meristematic activity as measured by tillering and leaf production is dependent to a large extent upon nitrogen supply, the relation between nitrogen uptake and tillering and leaf growth may now be examined.

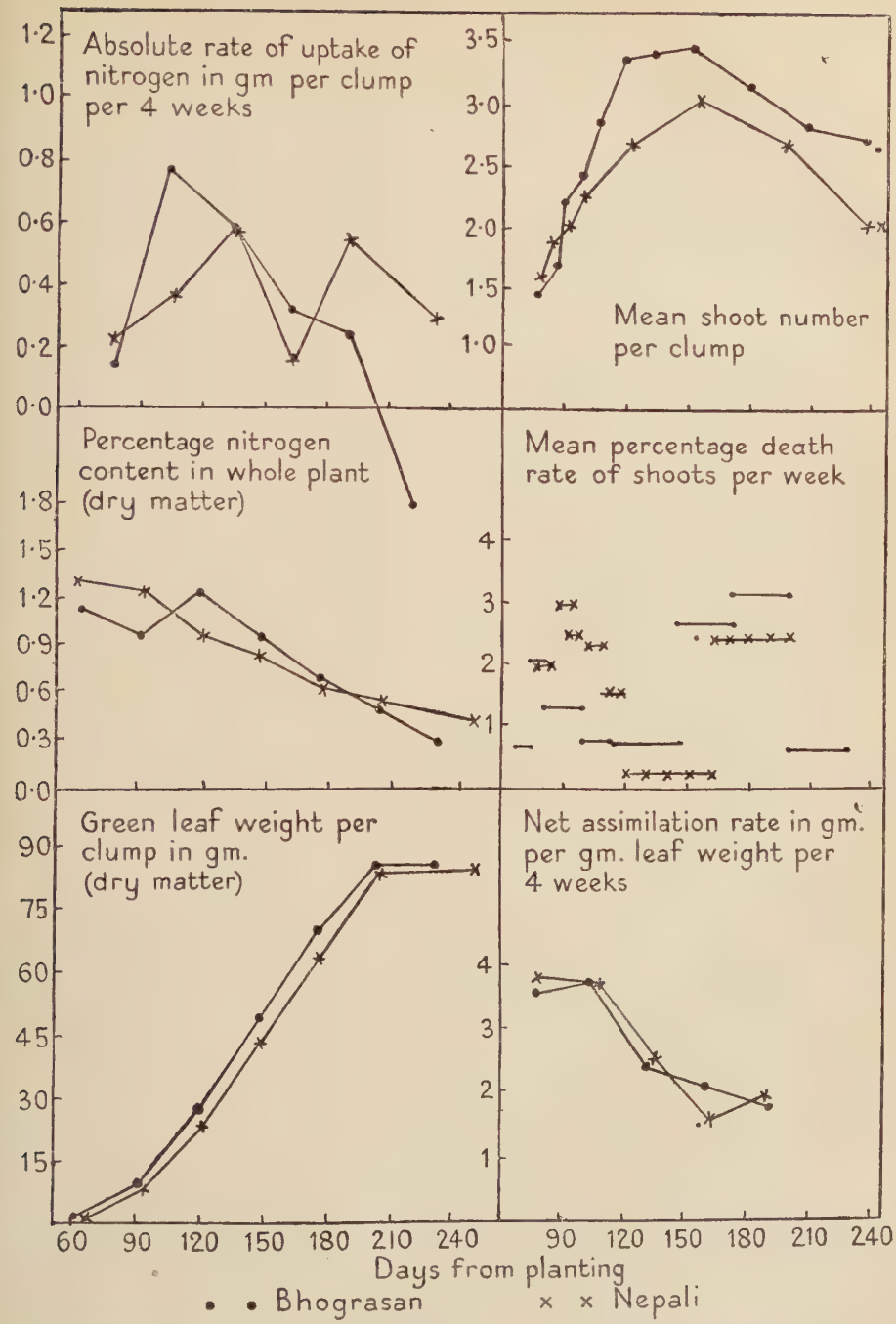
Absolute rate of uptake of nitrogen in gm per clump per 4 weeks
1·2
1·0
0·8
0·6
0·4
0·2
0·0
3·5
3·0
2·5
2·0
1·5
1·0
Mean shoot number per clump
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
4
3
2
1
Green leaf weight per clump in gm. (dry matter)
90
75
60
45
30
15
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
4
3
2
1
60 90 120 150 180 210 240
90 120 150 180 210 240
Days from planting
• • Bhograsan
× × Nepali

FIG. 1.

The absolute rate of uptake of nitrogen (Fig. 1) in Bhograsan reached maximum level by 15 weeks, then gradually diminished and fell to zero by 30 weeks. In Nepali the rate of uptake of nitrogen rose to maximum (though to a lower level as compared to Bhograsan) by about 19 weeks, then diminished gradually until about 23 weeks, and then again rose to a secondary maximum. Up to the first 13 weeks the rate of uptake of nitrogen was more or less identical in both Nepali and Bhograsan, which correlated with more or less similar tillering rate and leaf growth in both. Subsequently the slopes of the respective curves of uptake of nitrogen diverged considerably and also those of the tillering and leaf-weight curves. The maximum difference in tillering at 17 weeks was apparently conditioned by maximum difference in uptake rate between 13 and 17 weeks.

During the first 13 weeks the nitrogen content was higher in Nepali. During the succeeding 12 weeks—13th to 25th—the rate of uptake of nitrogen as well as percentage nitrogen content was on the whole higher in Bhograsan, which may be interpreted as indicating greater availability of nitrogen in Bhograsan during this period and also that nitrogen limited growth in Nepali. After 25 weeks nitrogen appeared to be increasingly available in Nepali, as indicated by the rise in the rate of uptake as well as increase in the rate of leaf growth, with the result that final leaf weight was almost as high as that in Bhograsan. The final yield was, however, lower in Nepali because, although the crop made up in growth at a later stage, this growth was confined to individual stalks and not to increase in their number, the tillering phase having reached its peak earlier. It may be noted that the net assimilation rate was more or less identical in the two cases and thus in no way affected growth in Nepali.

The weather data given in Table II indicate that there was no heavy rain between the 13th and the 25th week to account for washing down of nitrate below root depth when nitrogen appeared to be limiting growth in Nepali. With the data at hand it cannot be conjectured how nitrogen became deficient between 13 and 25 weeks and relatively more available subsequently. In any case the growth analysis indicated the operation of some adverse soil factor in Nepali which was situated only a few yards away from Bhograsan. Bhograsan is also noted at Pusa for its fertility.

TABLE II

Weather Data

Days from planting.		Rainfall in inches.		Mean temperature in °F. Maximum.		Minimum.	
		N*	B*	N*	B*	N*	B*
0–91	Nitrogen not limiting	2·80	1·42	92·9	92·6	67·7	66·6
91–105	Nitrogen limiting	1·75	2·09	97·6	98·5	78·6	77·5
105–75	Nitrogen limiting	17·71	18·12	91·7	92·7	78·8	79·4
175–210	Nitrogen available	8·84	8·22	89·7	91·2	78·9	78·3

* N for Nepali; B for Bhograsan.

Bhograsan and Harpur Jhilli

Harpur Jhilli was ahead of Bhograsan as regards tillering for the first 13 weeks. The Harpur Jhilli crop was planted earlier by about 2 weeks and it is possible that it thus got a better start due to milder weather during this period. After this time the rate of tillering in Harpur Jhilli decreased rather suddenly. It may be noted, however, that a day before the next shoot-count was taken the crop was earthed up and, therefore, very likely a large number of young tillers were buried and could not be counted. Assuming that the same steady rate of increase continued, the mean number of shoots per clump would have been about three by the 15th week and therefore still above that in Bhograsan. Soon after, however, tillering in Bhograsan surpassed that in Harpur Jhilli, and a sharp break in the curve of tillering in the former occurred approximately by the 17th week. The crop was earthed up 3 days after this shoot-count, and there was only a slight increase in tillering after this time. After the 15th week, when tillering in Bhograsan overtook and surpassed that in Harpur Jhilli, it may be noted that the latter did not suffer from higher death-rate. In fact the death-rate was slightly greater in Bhograsan, but the production rate was much higher. The difference in mature canes at harvest was practically the same as that for the maximum tiller number.

Maximum green-leaf weight was attained in Bhograsan by 29 weeks and in Harpur Jhilli by 25 weeks. Leaf weight was, however, higher in Harpur Jhilli from the beginning until about 21 weeks. After this time it increased very little in Harpur Jhilli, but at a considerable rate in Bhograsan, with the result that the maximum leaf weight was higher in the latter.

For the first 13 weeks the rate of uptake of nitrogen as well as the percentage nitrogen content was higher in Harpur Jhilli. Tillering as well as leaf growth was maintained at a higher level. Between 13 and 17 weeks the rate of uptake of nitrogen was only slightly greater in Harpur Jhilli, whereas the percentage content decreased and almost equalized with that in Bhograsan. It would seem that soil nitrogen supply had become relatively deficient in Harpur Jhilli during this period. The falling off in tillering after the 15th week in Harpur Jhilli may also be noted in this connexion. After 17 weeks the rate of uptake was consistently lower in Harpur Jhilli and the uptake also ceased altogether much earlier, which accounts for the much earlier cessation of leaf growth.

It may be considered why soil nitrogen in Harpur Jhilli became relatively in short supply from the 13th week onwards. Harpur Jhilli being situated in a low-lying area, the soil got quickly saturated with moisture after the onset of the monsoon and remained so throughout the rainy season and even some time later. A green scum of algal growth could also be seen on the surface throughout the rainy season. It was at times difficult to walk between the rows for observation purposes on account of the slush, and hence the paucity of observations on tillering after the 15th week. Data on rainfall given in Table III indicate that from the 10th week onward there was a substantially heavier rainfall in the case of Harpur Jhilli which might have washed down soil nitrate. Alternatively, due to the excessive accumulation of soil moisture,

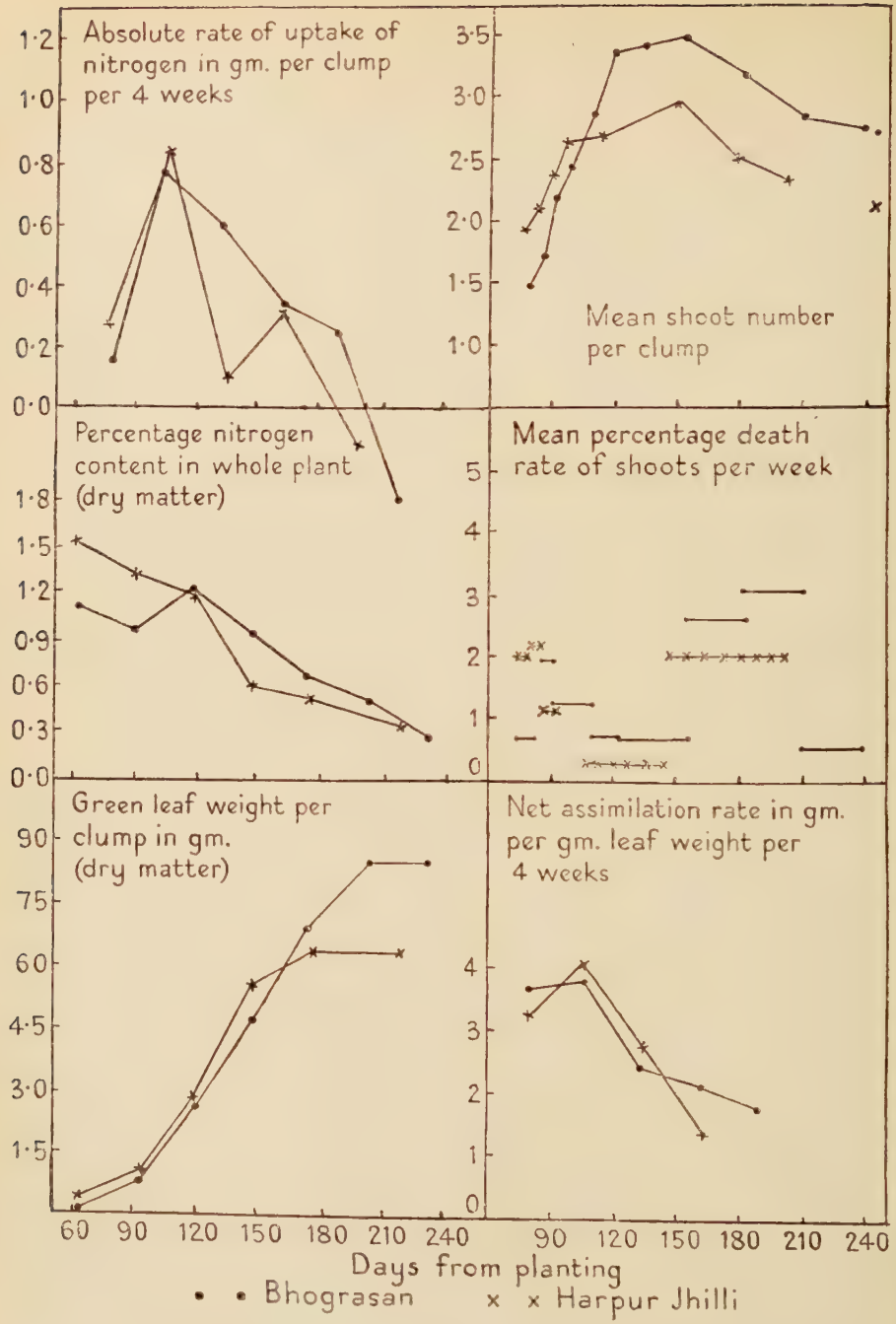
Absolute rate of uptake of nitrogen in gm. per clump per 4 weeks
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Mean shoot number per clump
3·5
3·0
2·5
2·0
1·5
1·0
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
5
4
3
2
1
0
Green leaf weight per clump in gm. (dry matter)
90
75
60
4·5
3·0
1·5
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
4
3
2
1
0
60 90 120 150 180 210 240
90 120 150 180 210 240
Days from planting
• • Bhograsan x x Harpur Jhilli

FIG. 2.

nitrification or absorption capacity of roots might have been inhibited. It is also possible that all these factors might have operated together or singly during this period. Be that as it may, it is clear that due to some soil condition comparatively less nitrogen was available to the Harpur Jhilli crop from the 13th week onwards.

TABLE III

Rainfall in Inches

	Time in weeks from planting				
	9th	10th	13th	14th	15th
Harpur Jhilli . . .	0·13	1·07	0·70	2·05	1·70
Bhograsan . . .	0·13	0·07	0·00	0·00	2·09

The other growth data are compared in Table IV. It will be seen that shoot growth in Harpur Jhilli was more rapid in the early stages. Cane formation also commenced earlier, as indicated by the proportion of stem weight in the whole plant. This earlier start in growth may be ascribed to better nitrogen and moisture status of the soil. Subsequently accumulation of moisture limited nitrogen supply and slowed down tillering, but did not apparently affect extension phase of growth of the parts already differentiated. It may also be noted that the net assimilation rate in Harpur Jhilli was relatively low between 21 and 25 weeks. Thus by 25 weeks practically the same amount of dry matter accumulated in both cases, as illustrated by the data in Table V, although its distribution among various parts of the plant was different.

TABLE IV

Growth Data, Heights of Plants (in inches)

Harpur Jhilli				Bhograsan			
Days after planting.	Plant height* (in.).	Stem height† (in.).	$\frac{\text{Stem weight}}{\text{Total weight}} \times 100$	Days after planting.	Plant height* (in.).	Stem height† (in.).	$\frac{\text{Stem weight}}{\text{Total weight}} \times 100$
64	7·8	—	—	63	7·0	—	—
92	12·5	—	—	91	10·4	—	—
120	24·7	—	—	119	18·9	—	—
148	—	23·7	38·0	147	32·9	14·4	29·0
176	—	40·3	50·0	175	—	27·8	43·0
				203	—	45·7	50·0
218	—	57·0	60·2	231	—	59·8	58·0

* Main shoot only. † All canes in a clump.

TABLE V

Accumulation of Nitrogen and Dry Weight

	Harpur Jhilli. (g.)	Bhograsan. (g.)
Total nitrogen content per clump	1·62	1·93
Total dry weight	306·6	292·9
Green leaf weight	67·8	70·0
Stem weight	153·4	126·2

After 25 weeks little leaf growth was made in Harpur Jhilli and a substantial difference in growth was established in the two fields after this time. Thus the Bhograsan crop outyielded the Harpur Jhilli crop mainly by virtue of its higher tiller number. It may be concluded that tillering in Harpur Jhilli was mainly depressed by nitrogen deficiency and the subsequent growth was checked by nitrogen shortage as well as other adverse conditions in the soil brought about by saturation with moisture.

Bhograsan and Brickfield

Although in the early stages the rate of tiller production was slightly higher in Brickfield (Fig. 3) the tiller number was practically equalized in the two cases by the 13th week. From this time until the 17th week tiller number was slightly higher in Bhograsan. In Brickfield tiller number actually decreased between 17 and 22 weeks due to lower production rate as well as a slightly higher death-rate. Between 22 and 26 weeks mortality was considerably greater in Bhograsan, so that by the end of the period the mean number of living shoots per clump was about the same in both. In spite of this the mean number of canes per clump at harvest was, however, significantly greater in Bhograsan. That this difference was not due to greater mortality in Brickfield can be seen from Table VI. Evidently cane formation was delayed in Brickfield.

TABLE VI

Numbers of Living and Dead Shoots

Brickfield				Bhograsan			
Date.	Total number of shoots per $\frac{1}{20}$ acre.	Dead hearts.	Living shoots.	Date.	Total number of shoots per $\frac{1}{20}$ acre.	Dead hearts.	Living shoots.
4/10	2,599	558	2,041	17/10	3,021	912	2,109
27/10	(Mature canes)	—	1,465	17/10	(Mature canes)	—	2,066
21/12	(,,)	—	1,524	4/12	(,,)	—	2,071

Maximum weight of green leaf was attained in both at about the same time, although the maximum levels were very different (Fig. 3). By about 13 weeks leaf weight in Bhograsan showed an increase over Brickfield and the difference widened further until about 20 weeks. Between 20 and 24 weeks the rate of leaf growth in Brickfield was higher than in Bhograsan, but subsequently it fell behind considerably in the former.

The absolute rate of uptake of nitrogen was practically indentical in both for the first 13 weeks or so, although the percentage nitrogen content was appreciably higher in Brickfield. Between 13 and 17 weeks, when the percentage nitrogen content was still higher, the uptake rate was lower in Brickfield. Evidently growth in Brickfield was restricted by some factor other that nitrogen and therefore nitrogen accumulated. Between 17 and 20 weeks growth in Brickfield diminished still further as compared to Bhograsan as indicated by the smaller increase in leaf weight and decrease in tiller number.

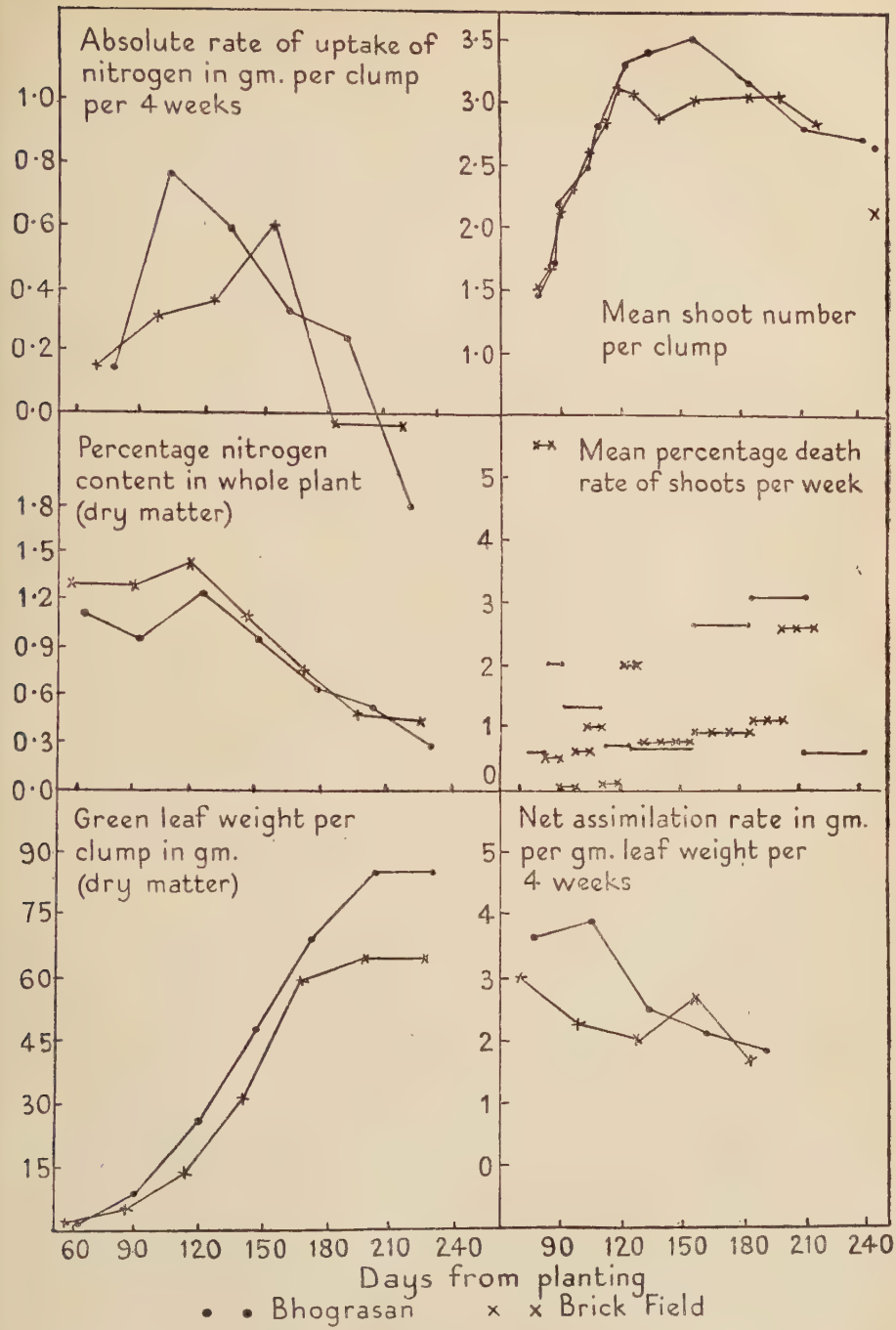
Absolute rate of uptake of nitrogen in gm. per clump per 4 weeks
1·0
0·8
0·6
0·4
0·2
0·0
3·5
3·0
2·5
2·0
1·5
1·0
Mean shoot number per clump
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
5
4
3
2
1
0
Mean percentage death rate of shoots per week
Green leaf weight per clump in gm. (dry matter)
90
75
60
45
30
15
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
5
4
3
2
1
0
60 90 120 150 180 210 240
90 120 150 180 210 240
Days from planting
• • Bhograsan x x Brick Field

FIG. 3.

During this period the uptake of nitrogen was also lower and the percentage nitrogen content higher in Brickfield, which again indicates that some factor other than nitrogen limited growth. Between 20 and 25 weeks growth improved in Brickfield, the rate of uptake of nitrogen also increased, and the percentage nitrogen content was about the same as in Bhograsan or slightly higher; this indicates that the factor that limited growth was no longer operative. From the 25th week onwards leaf growth in Brickfield was very poor as compared to Bhograsan, and this appears to be related to the much earlier cessation of nitrogen uptake.

From Fig. 3 it will be seen that the net assimilation rate was, on the whole, lower in Brickfield up to about 20 weeks.

From the data on rainfall given in Table VII it will be seen that March, April, and the first half of May during 1945 were dry as compared to 1944. During this period growth, however, did not suffer in Brickfield, probably because the soil moisture was just sufficient for the early stages of the crop. As already pointed out, growth in Brickfield lagged behind that in Bhograsan

TABLE VII

Rainfall Data in Inches per Month

	March.	April.	May.	June.	July.
Rainfall in 1944 . .	0·86	0·54	2·09	5·44	7·01
Rainfall in 1945 . .	0·00	0·00	1·68	4·37	3·80

from the 13th week onwards until the 20th week, and this would seem to be accounted for by low rainfall during this period in 1945. Data in Table VIII indicate that soil moisture did not increase during July 1945 as it usually does after the hot weather, and it is conceivable that growth in Brickfield was checked between 13 and 20 weeks by the comparatively droughty conditions.

TABLE VIII

Percentage Moisture Content of Soil

	Brickfield		Bhograsan	
Date	1/6/45	25/7/45	11/5/44	6/7/44
	11·1%	11·1%	8·0%	15·6%

From the data in Table IX it will be seen that the effect of partial drought was also reflected in the moisture content of the stem, particularly in the July sample.

TABLE IX

Moisture Content of Stem as Percentage of Dry Weight

Date.	Bhograsan.	Date.	Brickfield.
26/5	291	28/5	398
21/6	527	25/6	483
19/7	600	22/7	406
16/8	506	21/8	558

The data on the development of the cane given in Table X are also illuminating in this connexion. By about 25 weeks the number of internodes (only fully elongated internodes were considered) was indeed greater by about 1 in Brickfield, which indicates that at least nitrogen did not limit growth in the early stages. The mean internodal length was, however, shorter by 1·7 in., which points to the extension phase of growth being limited by moisture supply. Subsequently the number of internodes differentiated was much less in Brickfield, thus indicating deficiency of nitrogen. The very small increase in leaf growth and the cessation of nitrogen uptake after about 24 weeks has already been referred to. It is significant that after 25 weeks the differences in internodal length consistently diminished, thus indicating that soil moisture was no longer limiting the growth of internodes that developed later. Indeed it seems plausible that the increasing saturation of the heavy soil in Brickfield made soil nitrogen less available to the crop in the later stage.

TABLE X

Number and Length of Internodes

Bhograsan			Brickfield		
Days from sowing.	Mean internode number.	Mean internodal length (in.).	Days from sowing.	Mean internode number.	Mean internodal length (in.).
175	7·4	3·9	170	8·3	2·2
203	11·1	4·1	198	10·2	2·9
231	13·4	4·4	226	11·9	3·4

The much smaller percentage of cane formation in Brickfield can now be understood. By 20 weeks the shoot number in Brickfield was substantially reduced due to deficient moisture supply. Subsequently although the shoot number was equalized, due to higher death-rate in Bhograsan and increase in Brickfield, the latter crop was more heterogeneous, as it consisted of a relatively greater proportion of tillers that developed later. The drought also adversely affected cane formation in the older shoots, as indicated by the smaller rate of increase in plant height and the lower percentage of stem weight in total plant weight.

To sum up: Growth in Brickfield up to the end of July was limited by the comparatively dry weather and the consequent restricted soil-moisture supply which adversely affected leaf-growth, assimilation rate, and development of cane. As a result, although the rate of uptake of nitrogen was diminished, the percentage nitrogen content was higher in Brickfield. Conditions regarding soil-moisture supply then improved and so also did growth. Later, however, nitrogen limited growth, possibly due to excessive accumulation of soil moisture which reduced the supply of available nitrogen. The final yield of millable canes was therefore reduced, due to decrease both in its number and size.

Bhograsan and North Pangarbi

For the first 14 weeks or so tiller production rate was on the whole higher in North Pangarbi. Since dead hearts were removed from North Pangarbi during this period, the mortality rate could not be calculated. Between 14 and 17 weeks tiller production rate was still higher, but the death-rate was also considerably higher, and thus tiller number was more or less equalized in both. Between 17 and 20 weeks higher mortality rate reduced the tiller number in North Pangarbi. Between 20 and 25 weeks although the death-rate was higher in North Pangarbi the production rate was still greater as compared to Bhograsan and the tiller number again equalized. No data were collected from 25 weeks onwards till harvest due to political disturbances during 1942 and lodging in the other treatments as a result of heavy rain and storm in the last week of September.

There was more of leaf growth in North Pangarbi during the first 14 weeks. The leaf-growth rate declined appreciably in North Pangarbi during the next 5 weeks or so. Between 19 and 22 weeks leaf-growth rate was more or less identical in the two fields. Due to political disturbances during August 1942 the sample at the proper time could not be collected. Considering, therefore, the period between 22 and 30 weeks it is seen that the leaf growth rate during this period was considerably slower as compared to Bhograsan, but subsequently it continued at a faster rate.

The percentage nitrogen content was more or less similar in the two cases up to about 22 weeks, and subsequently it appeared to be slightly higher in North Pangarbi. The rate of uptake was higher in North Pangarbi during the first 14 weeks. It has already been seen that tiller as well as leaf growth was higher in North Pangarbi during this period. From 14 to 30 weeks the uptake rate was consistently lower in North Pangarbi, although the percentage nitrogen content did not differ from that in Bhograsan. Distribution of rainfall and other growth data in Table XI indicate a fairly satisfactory correlation between distribution of rainfall and growth, and it seems permissible to conclude that

TABLE XI

Effect of Rainfall in Inches

Period.	Month.	Rainfall		Remarks.
		Bhograsan.	N. Pangarbi.	
First 14 weeks	March	0·66	0·86	Leaf growth and tillering superior in N. Pangarbi.
	April	0·22	0·54	
	May	1·32	0·00	
15th to 19th week	—	4·15	2·10	Leaf growth rate reduced in N. Pangarbi.
20th to 22nd week	—	8·48	9·27	Leaf growth rate almost identical in both.
23rd to 30th week	August	9·28	5·99	Leaf growth rate considerably reduced in N. Pangarbi.
	September*	7·43	13·54	

* 8·6 inches fell about a week before the September sample was taken in N. Pangarbi.

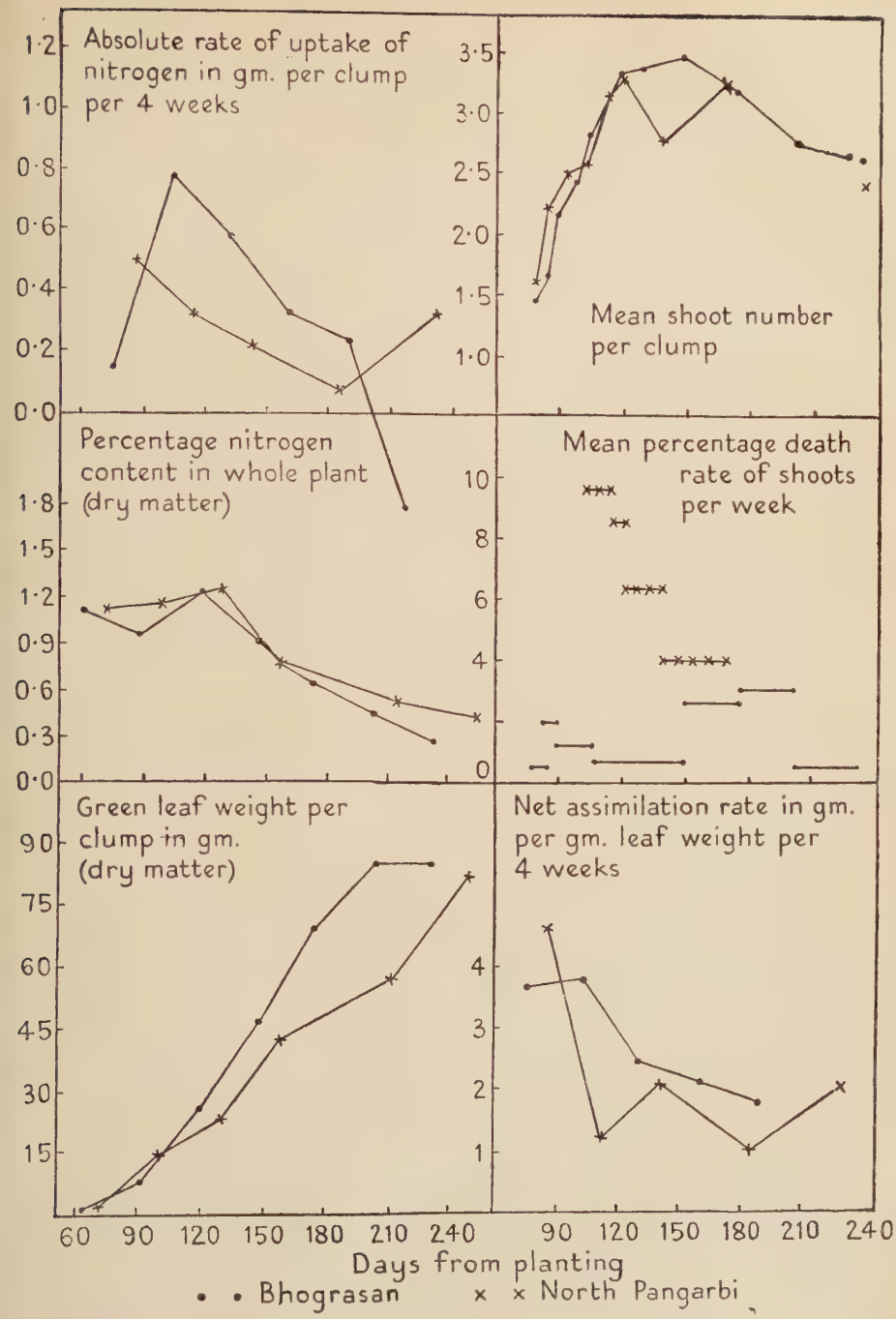
Absolute rate of uptake of nitrogen in gm. per clump per 4 weeks
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Mean shoot number per clump
3·5
3·0
2·5
2·0
1·5
1·0
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
10
8
6
4
0
Green leaf weight per clump in gm. (dry matter)
90
75
60
45
30
15
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
4
3
2
1
60 90 120 150 180 210 240
90 120 150 180 210 240
Days from planting
• • Bhograsan
× × North Pangarbi

FIG. 4.

the two setbacks in growth suffered by the North Pangarbi crop, viz. during May and August, were due to insufficient water-supply. The rates of elongation of shoots during the monsoon period also indicate the effect of deficient rainfall during August.

TABLE XII

Rates of Shoot-growth

Period.	North Pangarbi.	Period.	Bhograsan.
15/7–31/7	1·29 cm. per day	15/7–12/8	1·72 cm. per day.
31/7–12/8	1·11 ,, ,,	—	—
12/8–1/9	1·44 ,, ,,	12/8–5/9	1·34 cm. per day.
1/9–25/9	1·22 ,, ,,	5/9–4/10	1·43 ,, ,,

The very slow rate of shoot elongation, viz. 1·11 cm. per day during the first half of August in North Pangarbi, is worthy of note. It may also be noted that the difference in final height of the cane in favour of Bhograsan was in a large measure due to the longer internodal length in the latter case.

It is also interesting to note that the net assimilation rate was also lower in North Pangarbi between 15 and 19 weeks and also between 23 and 30 weeks, during which periods water-supply was the limiting factor.

Thus the yield in North Pangarbi suffered, largely due to poor growth of individual cane and, to some extent, to reduction in cane number.

Bhograsan and Chonia

In Chonia the percentage of germination was lower than in Bhograsan. In the 1943 kharif the field was put under maize and consequently the preparation of the field for the following sugar-cane crop was considerably delayed and the usual cultivation schedule was also not carried through. Even at the time of planting the surface soil consisted of clods, and it is felt that germination was comparatively poor on account of the unfavourable physical condition of the soil. Again, 2 weeks after planting there was intermittent rain throughout the succeeding 6 weeks (amounting to 1·40 in.) and this also added to the stiffness of the surface crust. Planting in Bhograsan was done 9 days earlier, and the Bhograsan soil being light, germination was not as adversely affected as in Chonia.

Tillering in Chonia lagged behind Bhograsan from the very beginning and the difference increased with time up to 17 weeks. During the first 13 weeks the production rate was higher and the death-rate lower in Bhograsan. Between 13 and 26 weeks rate of production and death of tillers were more or less identical in the two cases, and thus the difference in tiller number established during the first 13 weeks persisted. Between 26 and 30 weeks the death-rate in Bhograsan was higher and the difference in tiller number amounted almost to nil. Subsequently the death-rate in Chonia was slightly higher. The ultimate difference in the number of mature canes in favour of Bhograsan was, however, more or less proportional to the difference in germination percentage.

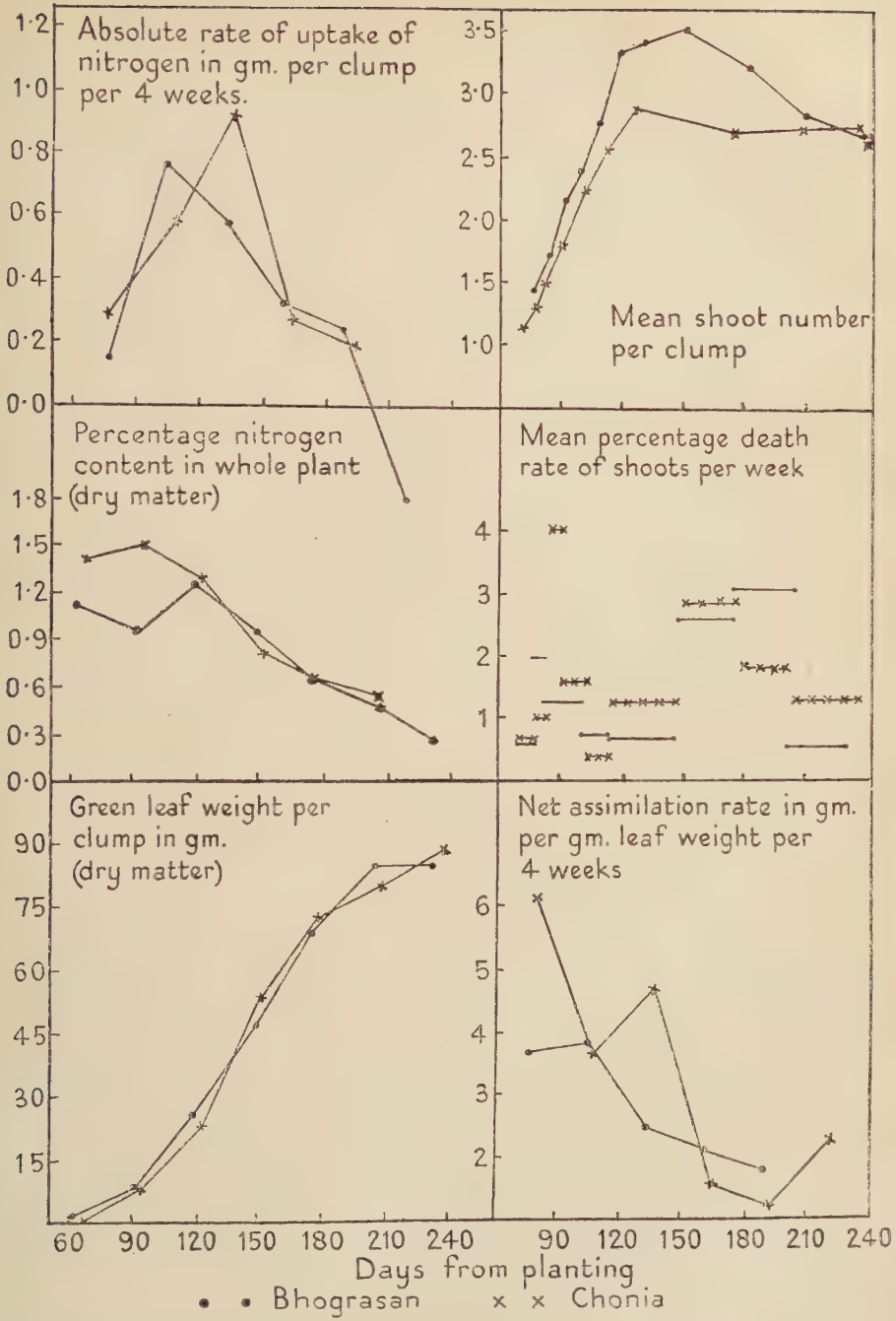
Absolute rate of uptake of nitrogen in gm. per clump per 4 weeks.
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Mean shoot number per clump
3·5
3·0
2·5
2·0
1·5
1·0
Percentage nitrogen content in whole plant (dry matter)
1·8
1·5
1·2
0·9
0·6
0·3
0·0
Mean percentage death rate of shoots per week
4
3
2
1
Green leaf weight per clump in gm. (dry matter)
90
75
60
45
30
15
Net assimilation rate in gm. per gm. leaf weight per 4 weeks
6
5
4
3
2
60 90 120 150 180 210 240
90 120 150 180 210 240
Days from planting
• • Bhograsan x x Chonia

Fig. 5.

Difference between leaf weights which was established early continued up to about 17 weeks, and equality was attained by about 20 weeks. Between 20 and 25 weeks the leaf weight increased at the same rate in the two cases. Between 25 and 29 weeks leaf growth was less in Chonia but subsequently it was greater, and thus the final leaf weight was more or less identical.

The rate of uptake of nitrogen as well as the percentage nitrogen content indicates that nitrogen was not the factor limiting leaf growth (during the first 17 weeks) and tillering. Very probably it was the stiff nature of the soil surface, referred to earlier, that suppressed emergence of tillers. This would seem to be borne out by the fact that both production and death-rates of tillers were lower during the first 13 weeks, as already noted above. Once, however, the plants were established their growth continued at the same rate as, or even higher than, that in Bhograsan.

Unfortunately the last sample in Chonia was accidentally destroyed by fire and its nitrogen content could not be estimated. From the general trend of the curve of nitrogen uptake and the percentage nitrogen content it appears, however, that nitrogen did not become unavailable at later stages as it happened with heavy soils in Harpur Jhilli and Brickfield. It may be noted that Chonia is not situated in a low-level area and therefore there is adequate drainage. Actually the average size and weight of an individual cane was larger in Chonia, but the total yield suffered because of the smaller number of plants per unit area as a result of poor germination and setback to tillering in the early stages.

Discussion

The outstanding effects of environmental conditions on growth of sugar-cane in the four seasons under consideration may be summarized as follows:

Field.	Developmental characters affected (as compared with Bhograsan).	Factors responsible.
Nepali . .	Tillering and final cane number reduced.	Deficiency of nitrogen in the early stages.
Harpur Jhilli .	Tillering, leaf growth, and final cane number reduced.	Deficiency of nitrogen after 13 weeks due to excessive soil moisture.
Brickfield .	Tillering, leaf and stem growth, net assimilation rate, and cane number and size reduced.	Drought in the early stages; deficiency of nitrogen later due to excessive soil moisture.
Chonia . .	Germination, tillering, and final cane number reduced.	Stiff surface crust due to insufficient preparation of land.
North Pangarbi	Reduction in leaf and stem growth, net assimilation rate, cane number, and size.	Partial drought in May and August.

Reduction in yield as compared to that of Bhograsan appears, therefore, to be due to (i) poor germination due to insufficient preparation of land, (ii) partial drought, (iii) deficiency of nitrogen, and (iv) unknown soil factor.

Factor (i) need not be considered.

As compared to Bhograsan, tiller production in both Nepali and Harpur Jhilli suffered in the early stages as a result of nitrogen deficiency, whereas in Brickfield and North Pangarbi tiller number was reduced later, due to partial drought. It would seem that both nitrogen and moisture influence tillering, but the latter apparently controls growth and survival of the tiller and not its initiation. Since difference in tiller number established as early as 17 weeks after planting reflect upon cane number at harvest, it is obvious that tillers developed during this period contribute substantially to yield; the possibility that the effects of borers might alter this relationship later is, however, not ruled out. The maximum tiller number is usually attained by the end of the hot weather (early July), but it does not appear likely that the usual high temperature and low relative humidity during this period have a markedly adverse effect upon tillering, at least in the case of Co 313. More likely, nitrogen has a relatively preponderant effect upon tillering. The possibility of tillering benefiting by irrigation (if possible) in the case of the usual hot and dry weather during this phase is not excluded, but the effect of increased soil moisture on the availability of nitrogen may also be involved.

With the onset of the monsoon and the accompanying increase in relative humidity stem internodes begin to elongate. At this stage demand for water increases considerably because water is required for the extension phase of growth as well as for the necessary turgidity of leaves for active assimilation. Deficient rainfall is, however, likely to affect adversely assimilation rate and also leaf and cane growth. The variety Co 313 appeared to be susceptible to drought at this stage, as it suffered in all these respects in North Pangarbi and Brickfield where drought occurred.

With uniform distribution of rainfall and in absence of waterlogging it would then seem that Pusa soils can produce per acre at least 20 tons of millable cane under the usual rotations and even without addition of nitrogen at the time of planting.

The importance of uniform distribution of rainfall raises the difficult issue of obtaining varieties more tolerant to partial drought than Co 313. It was seen how the net assimilation rate was depressed by the partial drought in Brickfield and North Pangarbi. It is therefore tentatively suggested that assimilation rate would serve as a sensitive and suitable index for testing tolerance of varieties to drought. In fact, the writer (1943 and 1948) has observed that in a few sugar-cane varieties that he had occasion to study in a preliminary way (though not from the view-point of drought tolerance) the assimilation rates (as estimated by Sach's half-leaf method and by difference between the total carbohydrate contents of morning and afternoon samples of leaves) differed appreciably. This observation accords with Watson's view (1947) that the net assimilation rate varies between varieties of the same species. It conflicts with Heath and Gregory's generalization (1938) about the constancy of the net assimilation rate. Watson (1947), however, accounts for the constancy of net assimilation rate, observed by Heath and Gregory, by

postulating that species of higher net assimilation rates may have been associated with less favourable environments.

Crowther (1934), in cotton, and Watson (1947), in wheat, sugar-beet, and potato, found that yield was more closely related to leaf area than to net assimilation rate. According to Crowther (1934 and 1937) nitrogen and water-supply had no effect on the net assimilation rate in cotton in the Sudan, whereas in Egypt both nitrogen and spacing affected it. Very likely the rate of carbon assimilation was not depressed on account of the leaf turgidity being little affected by the different levels of irrigation used. Crowther interpreted the effect in Egypt as due to differential shading brought about by nitrogen and spacing. Tiver and Williams (1943) found, on the other hand, that growth of the flax plant was depressed by low-moisture treatment due to a depression of the net assimilation rate. Petrie and Arthur (1943) also found a similar effect of drought in the case of the tobacco plant. To sum up, although observations, so far reported in the literature, on the infiuence of environmental factors on the net assimilation rate and on its relation to yield do not lead to a consistent view, it appears to the writer that a systematic study of the relation between assimilation rate of sugar-cane varieties and environmental conditions (particularly restricted moisture supply) might repay the effort.

It is conceivable that in spite of partial drought during July the Brickfield crop would have subsequently made up in growth had it not suffered from nitrogen deficiency due to excessive accumulation of soil moisture. In view of the existence of extensive low-lying areas in North Bihar it is worth considering whether varieties more tolerant (than Co 313) to this soil condition can be obtained. Since leaf growth was conspicuously checked under these conditions it appears necessary to ascertain, in the first instance, whether some adverse soil condition, for example, reduced aeration, checks the supply of available nitrogen or inhibits root activity. A comparative study of the rate of leaf production in different varieties under this condition may also prove useful in this connexion. It is worth considering, besides, whether a variety with a relatively smaller requirement of nitrogen, if there be any, might not be more suitable to these conditions.

In the case of Nepali nitrogen limited growth only during the first 25 weeks, and subsequently it was in sufficient supply. The analysis of weather data does not throw any light on this peculiar soil condition. It may be noted that nitrogen applied as castor cake (in nitrogen treatment in Nepali) increased tillering during the first 12 weeks, but subsequently its effect disappeared completely. From the data at hand it is not possible to suggest the nature of the factor that adversely affected soil nitrogen. A close study of soil conditions is indicated, for it is in such cases that ameliorative measures may be expected to raise yields by ensuring efficient utilization of nitrogen.

Summary

In North Bihar in north-east India is located the second largest white-sugar belt in India, which is unique in the respect that sugar cane is grown there as

an annual crop entirely under rain-fed conditions. The average yield of millable cane approximates to about 15 tons per acre, and response to manuring with nitrogen and phosphorus is inconsistent.

Results presented in the paper relate to developmental observations on the variety Co 313 carried out during four seasons in six different fields (unmanured control plots) at Pusa, where conditions are fairly representative of the tract. Data on growth in the field (Bhograsan) which gave the highest yield are taken as a base-line for comparison with growth in other fields. The relation of growth to variation in final yield is discussed.

It was observed that the tillering phase which is nearly completed (during the hot weather) by the time the monsoon sets in, and the intensity of which influences in a large measure the yield of mature cane, was not so much influenced by the intensity of the hot weather as by the nitrogen supply.

During protracted periods of scanty rainfall during the monsoon growth suffered as a result of depression in the rates of assimilation and leaf growth. It is suggested that for selecting varieties more tolerant than Co 313 to partial drought the assimilation rate might serve as a sensitive and suitable index.

Waterlogging in low-lying areas also checked growth by depressing the rate of uptake of nitrogen and leaf growth. It is suggested that further investigation on the influence of waterlogging on the availability of nitrogen, and on root activity and rate of leaf production in different varieties, might point to a suitable index for selecting varieties adapted to this condition.

Growth analysis also pointed to another soil condition, not identified as yet, under which availability of nitrogen was temporarily reduced. It is believed that its identification might indicate the means of ensuring more efficient utilization of nitrogen.

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Physiological and Ecological Studies in the Analysis of Plant Environment

V. An Assessment of the Factors controlling the Distribution of the Bluebell (*Scilla non-scripta*) in Different Communities

BY

G. E. BLACKMAN

(*Department of Agriculture, Oxford*)

AND

A. J. RUTTER

(*Department of Botany, Imperial College, London*)

With five Figures in the Text

INTRODUCTION

THE four previous papers in this series (Blackman and Rutter, 1946, 1947, 1948, 1949) have been concerned essentially with an analysis of the relative importance of the light factor and the levels of mineral nutrient supply in determining the distribution and growth of the bluebell (*Scilla non-scripta*) in woodland. It has been demonstrated for a number of woodland sites that where the canopy is closed the variations in bluebell density are largely determined by the overall seasonal differences in the degree of shading. Subsequently it was shown by field and pot experiments, where the light intensity was varied by means of screens, that the bluebell does not demand a high status of mineral nutrition and can in no way be regarded as an 'obligate shade' plant in which the assimilation rate and growth rate reach maxima at low levels of light intensity. In the first place, over the range of 0·1 to full daylight, the net assimilation rate is proportional to the logarithm of the light intensity. Secondly, though the leaf-area ratio increases in logarithmic

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proportion to the degree of shading, the growth rate does not approach the maximum until the light intensity exceeds 0·6 daylight. It is therefore clear that in woodland the light intensity is likely to be the major factor controlling growth. In fact, so far as conditions of shade are concerned, the bluebell reacts like a typical 'sun' plant.

In the present paper an attempt has been made to assess the importance of factors other than light intensity which may play a part in controlling the distribution of *Scilla non-scripta*, both within and without woodland. In such an assessment many problems are involved and a certain diffuseness cannot be avoided. It is inevitable that the lines of investigations which have been undertaken are diverse and have arisen either out of the early light experiments or from a general consideration of the autecology of *S. non-scripta.*

Although the bluebell is a very common woodland plant, yet in some areas in the south-west of England, in Wales, and on the western coast of Scotland, *S. non-scripta* is often a component of grassland communities, and is also found in association with *Pteridium aquilinum*. This occurrence in such open situations has hitherto been explained on the basis that the sites had been woodland in the past, and that the bluebells have survived the felling of the trees. On the other hand, apart from the evidence of the present investigations, other observations cast doubt on this explanation. In islands off both the Brittany and Welsh coasts, bluebells can be found growing on rock ledges and in grassland and bracken communities where the shallowness of the soil and the full exposure to the wind could not have allowed tree growth in the past. Similarly, *S. non-scripta* is also found at altitudes well above the existing tree line, e.g. at 1,800–2,000 ft. in Wales (Cader Idris), the Lake District, and in Scotland (Ardgarten Forest).[1]

Against this background it seemed evident that the factors controlling the growth of *S. non-scripta* in grassland ought to be investigated. In considering the design of the experiments it appeared significant that at least in many of the localities examined the areas where the bluebells grew in the open were either not grazed or only very lightly grazed. This conjunction suggested that biotic factors of trampling or grazing might limit the distribution in such communities.

In the previous investigations no consideration was given to the possibility that in different communities ecotypes of *S. non-scripta* might have arisen, and that these types would exhibit different responses to the major factors controlling growth. Accordingly, comparative experiments have been made with bulbs collected from a number of contrasting habitats, in order that any differential effects of varying light intensity on growth and development could be studied and any potential difference in the ability to compete with grasses assessed.

A further problem which has been given detailed consideration relates to the possibility that when plants are grown under varying conditions of light intensity or with different levels in the nutrient status of the soil, then the

[1] Private communications from Dr. J. H. Salter, Dr. Muir, and Prof. T. G. Tutin.

effects of these variables may carry over into the *subsequent* year and cause variations in seasonal growth. The analysis of this problem has in turn necessitated a study of the relationship between seasonal growth rate and varying bulb size.

The present paper, therefore, consists in the main of four aspects relating to the analysis of the autecology of *S. non-scripta*. In the case of the experiments concerned with the nature of the competition with grasses and the comparison of ecotypes, the results cannot be differentiated since bulbs from different communities have been used in the grassland experiments.

Experimental Results

I. *The Nature of the Competition in Grassland*

In considering the nature of the competition between *S. non-scripta* and the constituent species of grassland communities it seemed probable that the major factors involved would vary with the dominant species of grasses in the sward. In consequence, during the two years in which experiments were carried out on an alluvial gravel soil at the Field Station, Slough, Bucks., the bulbs were planted in almost pure stands of different grass species which had been sown 3 or 4 years earlier. In 1939 the three swards were dominated by *Phleum pratense*, *Festuca pratensis*, and *F. rubra* respectively. In each sward dormant bulbs of widely varying sizes were planted in the autumn. The large bulbs had already reached a size when inflorescences are normally produced, while the small bulbs were estimated to be not more than 2 or 3 years old.

The method of planting the mature bulbs was to cut with a metal tube a cylindrical core from the turf, 2 in. in diameter and 4 in. deep. The bulb was placed in the bottom of the hole, and the core of turf replaced and pressed down. For the small bulbs a core of turf 1 in. in diameter and 2 in. deep was cut out; it was broken about $\frac{1}{2}$ in. below the top and the bulb was put between the two pieces. These pieces were then placed together again and the whole was slid into a piece of glass tube, which was open at each end, also 1 in. in diameter and 1–2 in. long. The tube containing the turf and the bulb was dropped into the hole in the plot; the turf was pressed well down, but the glass allowed to project 0·5–1·0 in. above the level of the ground. This procedure was adopted so that during the course of the experiment the small bulbs could be located, for without some method of marking it would have been almost impossible to find the bulbs at the end of the growing season when the leaves had died back. Moreover, the tubes facilitated the cutting of the grass without defoliating the bluebell plants. On general considerations it seemed unlikely that the presence of the tubes would introduce serious errors.

The success with which bluebells can compete with grasses is likely to be related to the rate of growth and the ultimate height of the different species. Accordingly, attempts were made to alter the rate of growth and height in the following ways. Half the plots were treated with ammonium nitrate applied

in solution at a rate equivalent to 50 lb. of nitrogen per acre, while the others were left untreated. In addition half the plots were kept short by cutting while the other half were left to grow freely. When the grass was cut, care was taken not to cut or damage the bluebell leaves. Differential grazing like this differential cutting is not likely to occur in nature, but might if the bluebells were readily distinguished in the sward, and were unpalatable to the grazing animal. These two types of treatment were combined factorially, giving four treatments in all, viz. not cut plus nitrogen, not cut, cut plus nitrogen, cut. For each grass the treatments were in threefold replication. Unfortunately the mature bulbs were so badly attacked by *Penicillium cyclopeum* that they were discarded. But reliable data were obtained for the small bulbs, of which 10 were grown on each plot.

Without additional nitrogen there was no significant difference between cut and uncut plots (Table I), but in the plots which had received nitrogen, cutting the grass caused a significant increase in the growth of the bluebells. On the uncut plots the application of nitrogen significantly decreased the growth, but on the cut plots a significant increase in growth was brought about by nitrogen.

This interaction is highly significant, and it is put forward that in the uncut plots any positive effect of nitrogen in increasing the growth of the bluebell is outweighed by an indirect negative effect of nitrogen in increasing the growth and height of the grasses, and thereby adversely affecting the bluebell because of the increased shading. In consequence, it is only when there is little or no shading of *S. non-scripta* on the defoliated plots that nitrogen causes a gain in weight.

TABLE I

Experiment XI, 1939. The Interaction of Selective Cutting and Nitrogen Supply on the Seasonal Growth of Seedling Bulbs of Scilla non-scripta *in Grassland*

Dry weight of 10 bulbs at end of season (grammes)

	Nitrogen.	No nitrogen.	Mean.
Sward cut	0·776	0·640	0·708
Sward not cut	0·575	0·663	0·619
Significant difference ($P = 0{\cdot}05$) .	0·071		0·051

Since the effect of nitrogen is positive on the cut plots and negative on the uncut plots, the mean effects of nitrogen are small, but there are differences for the grass species. With *Phleum pratense*, while there is a marked increase due to nitrogen on the cut plots, there is little or no decrease on the uncut plots. In consequence the mean effect of nitrogen on *S. non-scripta* is significant in this species (Table II). The overall effect of grass species is not, however, significant.

The initial dry weight of the ten bulbs planted in the previous autumn was 0·279 g., and it is clear from Tables I and II that these very small plants have

TABLE II

Experiment XI, 1939. The Interaction of Selective Cutting, Nitrogen Supply, and the Dominant Species of Grass on the Seasonal Growth of Seedling Bulbs of Scilla non-scripta *in Grassland*

Dry weight of 10 bulbs at end of season (grammes)

Dominant species.	Sward cut.		Sward not cut.		Means of cutting treatments.	
	Nitrogen.	No nitrogen.	Nitrogen.	No nitrogen.	Nitrogen.	No nitrogen.
Phleum pratense	0·821	0·578	0·648	0·674	0·734	0·626
Festuca pratensis	0·774	0·747	0·522	0·646	0·648	0·697
Festuca rubra	0·735	0·594	0·554	0·669	0·643	0·631
Significant difference ($P = 0·05$)					0·088	

been able to double their weight, even under the greatest degree of competition which this experiment produced; that is to say, on plots which received nitrogen and were not cut.

Further experiments on competition with grasses were carried out in 1940 on swards consisting either of *Dactylis glomerata* or *F. rubra* sown 4 years previously. In each experiment there were three treatments. Bulbs were either planted in the sward or in the soil after the surface turf had been stripped, while subsequently half the sward plots received ammonium nitrate at a rate equivalent to 50 lb. of nitrogen per acre. It was therefore possible to compare the growth of plants with and without competition from either of the grasses.

In these experiments a comparison was also made of bulbs collected from three different sites, so that the possible differences in these populations might be investigated. It is proposed to refer to these populations as 'strains', since it will be shown subsequently that they differed significantly in a number of physiological characteristics. Strain I came from an open oak (*Quercus robur*) wood at Warfield, Berks., when there was a thin understorey of coppiced hazel (*Corylus avellana*); strain II was collected from an ash wood with a closed canopy at Gravetye, Kent, and strain III from a plantation of larch (*Larix japonica*) at Shurlock Row, Berks. Strains I and II were represented by two sizes of bulb, while those of strain III were very much smaller. Taking first the large bulbs, the initial mean weights for strains I and II were 1·38 and 1·28 g. per bulb, while the corresponding figures for the medium-sized bulbs were similar, viz. 0·37 g. The small bulbs of strain III averaged 0·037 g. On account of the discrepancies between the initial weights of the different classes of bulbs, the ratio of final to initial weight has been used as the measure of seasonal growth. On this basis it is seen from Table III that the differences between the three treatments are highly significant when the data are transferred to a logarithmic scale. Growth was least on the plots receiving nitrogen where the plants only maintained their initial weight, while the increase was greatest on the stripped plots, i.e. without competition from *D. glomerata*.

TABLE III

Experiment XII, 1940. Seasonal Growth of Scilla non-scripta *in a* Dactylis glomerata *Sward*

Treatment.	Ratio of final to initial weight.	
	Ratio.	Log. transformation.
Surface turf removed . .	1·664	1·180
Untreated sward . . .	1·386	1·118
Nitrogen added to sward . .	1·116	1·025
Significant difference (log. scale $P = 0{\cdot}05$)		0·082

There were also significant differences in the growth made by the different kinds of bulb (Table IV). On the grass plots the large-sized bulbs of both strains I and II failed to maintain, or only just maintained, their initial weight, and on the bared plots the large strain II bulbs gained little during the season. On the other hand, the medium-sized bulbs made more growth, probably because they did not set seed. The small bulbs made only a slight gain over their initial weight. In only one case did the plants double their initial weight, namely the medium-sized bulbs of strain I on the bared plots.

TABLE IV

Experiment XII, 1940. The Effect of Strain and Bulb Size on the Seasonal Growth of Scilla non-scripta *in a* Dactylis glomerata *Sward*

Source and bulb size.	Ratio of final to initial weight.				
	Turf removed.	Untreated sward.	Nitrogen added to sward.	All treatments.	All treatments (log. scale).
Strain I					
Large . .	1·57	1·09	0·96	1·21	1·061
Medium . .	2·74	1·76	1·62	2·04	1·283
Strain II					
Large . .	1·13	1·00	0·78	0·97	0·965
Medium . .	1·82	1·80	1·13	1·58	1·182
Strain III					
Small . .	1·06	1·28	1·09	1·14	1·047
Significant difference (log. scale $P = 0{\cdot}05$)					0·075

Experiment XIII was similar in design to expt. XII, save that the dominant grass was *F. rubra* in which medium-sized bulbs of strains I and II and small bulbs of strain III had been planted. On account of the limited area available, the replication was only threefold, and none of the effects are significant. The results, however, set out in Table V are similar to those of expt. XII. The growth of flowering bulbs is apparently better on bared soil than in the sward; on the former the plants just doubled their weight, but on the latter failed to do so. Under each set of conditions the small bulbs lost weight during the season.

TABLE V

Experiment XIII, 1940. Seasonal Growth of Scilla non-scripta *in a* Festuca rubra *Sward*

Strain of *S. non-scripta.*	Ratio of final to initial weight. Turf removed.	Untreated turf.	Nitrogen added to turf.
Strain I, medium-sized bulbs . .	2·28	1·49	1·67
Strain II, medium-sized bulbs . .	1·72	1·38	1·41
Strain III, small-sized bulbs . .	0·89	0·85	0·57

II. *The Effects of Defoliation and Trampling on the Seasonal Growth of the Bluebell*

In designing experiments to investigate the effects of grazing animals on the growth of the bluebell, there are clearly two main considerations. Firstly, animals may defoliate the plants and, secondly, if they are unpalatable, they may only trample on them. To simulate the effects of grazing, the leaves, and the inflorescence if present, were cut off with a pair of scissors. To imitate the effects of trampling it seemed that an approximation to the mechanical action of a hoof could best be achieved by walking backwards along the row of plants, placing the heel on top of each plant. In both sets of experiments the dormant bulbs were planted in the autumn on a gravel soil in rows some 12 in. apart, with 6 in. between each bulb.

The effects of defoliation on seasonal growth. Experiments were carried out at Slough in both 1939 and 1940. Experiment XIV was designed to assess the influence of the frequency and time of defoliation on the changes of weight during the period of seasonal growth. Some thirty mature bulbs were planted on each plot in the autumn of 1938, and in the following spring the leaves and the inflorescence, if present, were cut off (1) before the inflorescence had emerged above ground, (2) before the flower buds opened, and (3) when the plants were in full flower. In addition, there were two sets of double defoliation; the leaves were cut at stages 1 and 2 or 1 and 3.

TABLE VI

Experiment XIV, 1939. The Effects of Defoliation at Different Stages of Development on the Seasonal Growth of Scilla non-scripta

Stage of development at time of defoliation.	Final dry weight per bulb (g.).
Single defoliation	
(1) Before inflorescence emerges	2·09
(2) Before flower buds open	1·67
(3) At flowering	2·01
Double defoliation	
(1) Before inflorescence emerges and (2) before flowering .	1·08
(2) Before inflorescence emerges and (3) at flowering . .	1·26
Control	3·62
Significant difference ($P = 0·05$)	0·44

From the results of Table VI it is clear that all cutting treatments have markedly depressed growth. When the leaves are cut off, the bases continue to grow a little, but no new leaves are produced, and there is in consequence the greatest decrease in assimilatory tissue with the later cuttings. If the first cutting is followed by a second later in the season, the effect is even more pronounced.

The mean initial dry weight was 4·01 g. and on all the plots the bulbs were attacked by *P. cyclopeum*. In consequence, even the control plants showed a slight decrease in weight over the season, whereas on the evidence of other experiments they might have been expected to double their weight. The results, however, suggest that under more favourable circumstances the once-cut plants would have failed to increase much in weight, and that a double defoliation would certainly have caused a loss in weight.

In 1940 another similar experiment was carried out, but, in this case, single bulbs in pots were employed. The initial dry weight was calculated for each bulb separately, and the analysis of variance is based on the ratio of final to initial dry weight. The results are shown in Table VII.

TABLE VII

Experiment XV, 1940. The Effects of Defoliation at Different Stages of Development on the Seasonal Growth of Scilla non-scripta

Stage of development at time of defoliation.	Ratio of final to initial dry weight.
Single defoliation	
(1) Before inflorescence emerges	0·71
(2) Before flower buds open	0·89
(3) At flowering	1·10
Double defoliation	
(1) Before inflorescence emerges and (2) before flowering	0·77
(2) Before inflorescence emerges and (3) at flowering	0·69
Control	1·24
Significant difference ($P = 0·05$)	0·30

In addition to the severe effect of two cuttings, this experiment shows that the effect of a single cutting is greater the earlier it is made. The pots tended to dry out very rapidly and the effects of frequent wilting must be taken into account. This water shortage is reflected in the small increase in weight on the controls, but here again there was some attack by *P. cyclopeum*. A similar experiment was made in 1940 with bulbs planted in open ground, and the greater effect of an earlier defoliation is again shown in Table VIII, although the difference between the two times of cutting is not quite significant.

The effects of trampling. Three experiments on the relationship between the incidence and intensity of trampling were carried out during 1939 and 1940. In expt. XVII, as soon as the leaves were well above ground the appropriate plants were 'trampled' at 7-, 14-, 21-, and 28-day intervals. Unfortunately the incidence of *P. cyclopeum* was widespread and even on the control plots

TABLE VIII

Experiment XVI, 1939. The Effects of Defoliation on the Growth of Scilla non-scripta

	Final dry weight per bulb (g.).
Control	1·231
Leaves cut at ground level at emergence of inflorescence	0·636
„ „ „ „ late flowering stage	0·851
Significant difference ($P = 0·05$)	0·267

little growth was made during the season. Nevertheless, from the data given in Table IX it is clear that frequent trampling has had a very adverse effect.

TABLE IX

Experiment XVII, 1939. The Effects of the Frequency of Trampling on the Seasonal Growth of Scilla non-scripta

Treatment.	Final dry weight per bulb (g.).
Control	3·04
Trampled every 7 days	1·24
„ „ 14 „	2·19
„ „ 21 „	2·08
„ „ 28 „	2·32
Significant difference ($P = 0·05$)	0·43

In 1940 a similar experiment was undertaken in which there were two intensities of trampling, viz. trampling once in 10 days and trampling once in 20 days. Superimposed on these treatments were two stages at which trampling was initiated; in the first the trampling was begun soon after the appearance of the leaves, i.e. on May 8, and in the second it was started 20 days later. In addition, the plots were split so that bulbs of strains I and II could be included in the experiment.

TABLE X

Experiment XVIII, 1940. The Effects of Trampling on the Growth of Scilla non-scripta

Period of trampling.	Final dry weight per bulb (g.). Intensity of trampling. Control.	10 days.	20 days.	Period means.	Period means (log. scale).
Trampling started May 8	—	0·583	0·695	0·726	0·86
„ „ „ 28	—	0·925	0·957	0·980	0·99
Intensity means	0·981	0·753	0·826	—	—
Intensity means, log. scale	1·00	0·86	0·91	—	—

Significant difference ($P = 0·05$) between period means on log. scale = 0·05
Significant difference ($P = 0·05$) between intensity means on log. scale = 0·06

Table X shows that both the intensity of trampling and the stage at which trampling is initiated produce significant effects on seasonal growth. The

deferment of trampling by 3 weeks has produced a smaller adverse effect. Since the mean initial weight in the previous autumn was 0·452 g., even the most intensive trampling has not prevented the plants from gaining weight during the season.

Although at first the bulb weights of strains I and II were similar (0·459 and 0·444 g.), by the end of the season there was evidence of an interaction between strain and the period of trampling. Extending the period has had a greater effect in the case of strain I (see Table XI). As strain I produces leaves earlier in the season than strain II (see p. 500), early trampling is likely to cause more damage to the plants with the most advanced leaf formation.

TABLE XI

Experiment XVIII, 1940. The Effects of the Period of Trampling on the Seasonal Growth of Two Strains of Scilla non-scripta

Trampling treatment.	Final dry weight per bulb (g.). Untransformed data.		Transformed data (log. scale).		Sig. diff. ($P = 0·05$).
	Strain I.	Strain II.	Strain I.	Strain II.	
Trampling started May 8	0·83	0·63	0·91	0·78	0·07
„ „ „ 28	1·19	0·77	1·09	0·89	
Mean	1·01	0·70	1·00	0·84	0·05

III. *The Differential Reaction to Varying Light Intensity of Bluebell Plants from Different Habitats*

The experimental methods adopted for the analysis of the effects of shading on the seasonal growth and development of the different strains of the bluebells were similar to those described in detail in the previous papers (Blackman and Rutter, 1947, 1948). Graded bulbs of uniform size were planted on a cultivated gravel soil in the autumn, and as soon as the leaves emerged above ground in the following spring the appropriate plots were shaded by means of light wooden frames covered either with butter-muslin or sheets of perforated zinc. During the growing season random samples were taken from each plot; the plants were divided up into bulbs plus roots, leaves, and inflorescence; the separate parts dried in an oven and weighed. Before the leaves were dried a sub-sample was taken for the determination of the ratio of leaf area to leaf weight and by means of this ratio the leaf area per plant was estimated.

From these data it is possible to calculate the effects of varying light intensity on the changes in plant weight, leaf-area ratio, and net assimilation rate of each strain.

In the largest of the experiments, viz. expt. V, 1940, besides three levels of light intensity (daylight and 0·68 and 0·22 daylight) there were eight nutrient treatments consisting of nitrogen, phosphorus, and potassium, alone and in combination, and three strains of bluebell. Only the interactions between the strains and varying light intensity will be considered in this paper, since the

mean effects of light intensity and nutrient supply have already been discussed (Blackman and Rutter, 1947, 1948) and all the strains have given a similar response to the nutrient treatments.

Two of the strains, I and II, have already been described on page 491, while the third strain (strain IV) consisted of bulbs purchased from a bulb farm in Gloucestershire, where the plants had been raised in the open. Because of the large number of bulbs required, it was not possible to ensure that the mean bulb weight at the time of planting in the autumn was exactly the same for each strain. The mean bulb weights of strains I and IV were similar—0·92 g.—but for strain II the weight was 0·79 g. To eliminate these initial differences two other methods of comparison were adopted. Firstly, over the period April 23 to May 28, that is shortly after the leaves had appeared to when the leaf area was at a maximum, the efficiency indices were compared. Secondly, the weights on July 17 relative to the weights at planting were examined. The results in Table XII and Fig. 1 show that there were considerable differences between the rates of growth of the strains; strain I had the highest and strain II the lowest rate.

TABLE XII

Experiment V, 1940. The Effects of Varying Light Intensity on the Growth Rate of Different Strains

	Percentage increase in dry weight per day, April 23–May 28.		
Light intensity.	Strain I.	Strain IV.	Strain II.
1·00 daylight . . .	3·32	3·01	3·03
0·68 „ . . .	3·32	3·05	2·95
0·22 „ . . .	2·30	1·64	1·61
Mean	2·98	2·57	2·53

Significant difference ($P = 0·05$) = 0·19

An analysis of variance of the data of Fig. 1 after conversion to a logarithmic scale reveals that the reactions to changes in light intensity do not differ significantly between strains.

A further experiment (expt. XIX) in which five strains were grown at four levels of light intensity was undertaken in 1940. In addition to the four strains already described, there was a fifth collected from a clearing in a beech wood at Yattendon, Berks., where there was a dense stand of coppiced hazel. The four light treatments were replicated five times, while 10 bulbs of each of the five strains constituted sub-plots. Each lot of 10 bulbs was weighed before planting in 1939, and the dry weights were calculated after the percentage dry matter of a sub-sample had been determined. The mean initial weights of 10 bulbs of the five strains were: strain I, 5·66±0·26 g.; strain II, 5·96±0·11 g.; strain III, 6·60±0·28 g.; strain IV, 6·22±0·09 g.; and strain V, 7·01±0·11 g.

All the bulbs were dug up on July 17, and an analysis of variance has been carried out on the ratio of the final to initial weight after transformation to a logarithmic scale. This analysis shows that a reduction from daylight to 0·68

of daylight caused no significant fall in the ratio over all five strains (2·76 as against 2·78), but that at the two lower light levels of 0·22 and 0·11 daylight, the mean ratios have been significantly depressed, viz. 2·10 and 1·53 respectively. As in the previous experiment, there were marked differences between the strains. Over all light intensities, strain V made the maximum growth (mean ratio 3·20), followed by strain I (2·71), strain III (2·24), strain II (1·74), and strain IV (1·69).

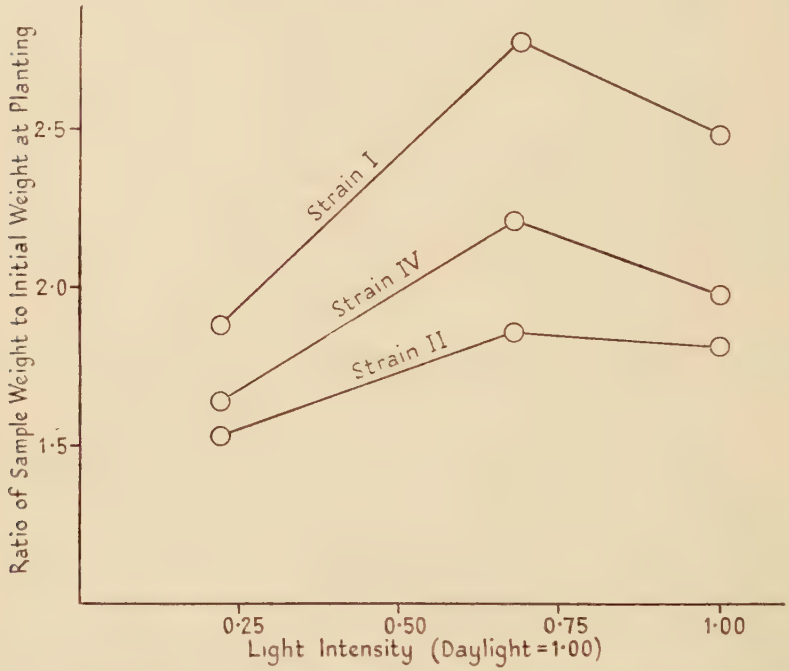

FIG. 1. Experiment V. The effects of varying light intensity on the seasonal growth of different strains of *Scilla non-scripta*.

This difference between the strains is brought out clearly in Fig. 2, where the changes in the ratio for each strain have been plotted against the logarithm of the light intensity. For strains I and III–V, curves of the form $y = a+bx+cx^2$ have been fitted to the data, but for strain II a better fit was obtained with a linear regression.

From these equations the extrapolated light values at which no growth would have been made during the season can be calculated. They are 0·087, 0·074, 0·071, 0·066, and 0·05 of daylight for strains IV, III, V, I, and II respectively.

A further analysis of the differences in the growth rate of strains can be obtained from the data of expt. V. It will be recalled that for the first sampling period besides estimates of the relative growth rates of the three strains under the different light intensities the leaf areas were also measured. It is

thus possible to calculate the corresponding net assimilation rates between the two sampling occasions and also the mean ratio of leaf area to plant weight—that is the leaf-area ratio of Briggs, Kidd, and West (1920). The assimilation rates have been calculated according to the usual formula (Gregory, 1926; Williams, 1946), while the leaf-area ratio is the arithmetic mean for the two

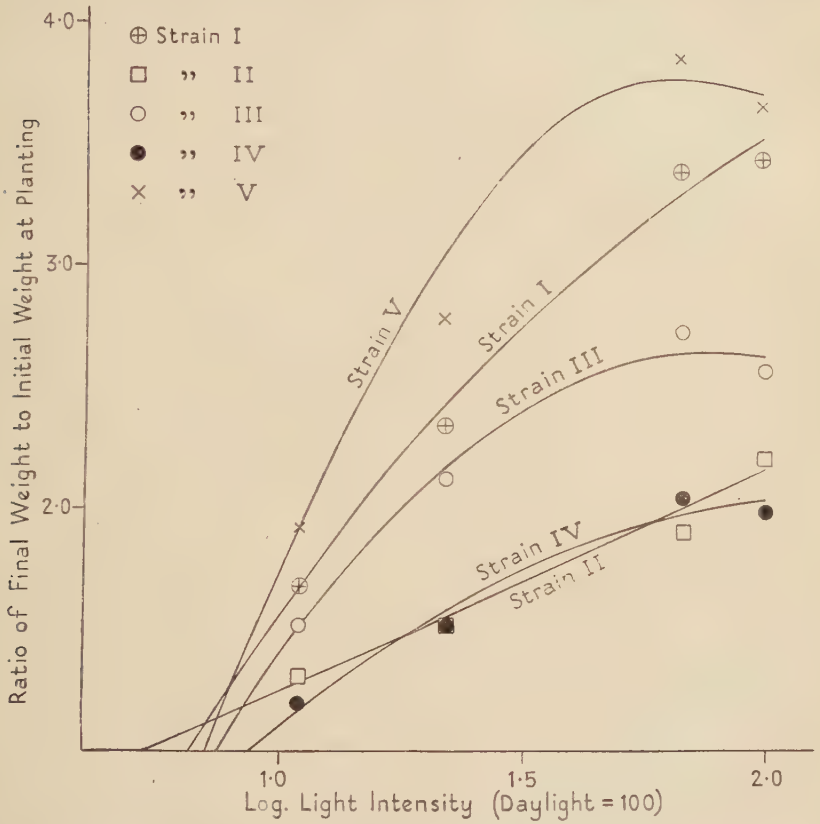

FIG. 2. Experiment XIX. The effects of varying light intensity on the seasonal growth of different strains of *Scilla non-scripta*: the observed means and fitted regressions are both given.

sampling occasions of the values of total leaf area divided by total plant weight, and is a measure of leafiness.

The relationships between strain, light intensity, net assimilation rate, and leaf-area ratio are shown in Fig. 3 *a* and *b*. In a previous paper (Blackman and Rutter, 1948) it was demonstrated that the net assimilation rate is linearly related to the logarithm of the light intensity, and statistical analysis shows that this relationship is not significantly different for the three strains. It is, however, clear from Fig. 3 *b* that there are significant differences in the leaf-area ratios of the three strains. It is also to be observed that for each strain there is a linear relationship between the ratio and the logarithm of light intensity.

Since relative growth rate is the product of net assimilation rate and leaf-area ratio, it follows that the differences in the growth rates of the strains shown in Table XII can be ascribed to the differences in the leaf-area ratios set out in Fig. 3 *b*, i.e. the greater leafiness of strain I as compared to strains II and IV.

A closer examination of these differences in leaf production reveals that the variation is linked with the date of leaf emergence in the early spring. By the time the plots were first sampled and shaded on April 23, strain I had a much larger relative leaf area than strain II, while strain IV was intermediate (see Table XIII). It is, however, apparent that by May 29 the differences between strains are less marked. In fact, there is now a significant interaction between strain and light intensity, for, whereas in full daylight strain II tended to be more leafy than the other strains, at the lower light intensities strain I is more leafy than strains II and IV.

TABLE XIII

Experiment V, 1940. Variations in Leaf Production by Strains and the Effects of Varying Light Intensity

	Leaf-area ratio ($cm.^2$ leaf per g. total plant weight). Strain I.	Strain IV.	Strain II.
Before shading (April 23)	40·6	35·9	29·6
Log. scale	0·604	0·546	0·460
Significant difference ($P = 0·05$) = 0·036			
After shading from April 23 to May 28			
Light intensity			
1·0 daylight	24·2	23·7	25·3
Log. scale	0·380	0·373	0·400
0·68 daylight	30·4	27·7	28·4
Log. scale	0·482	0·442	0·450
0·22 daylight	52·9	44·1	47·2
Log. scale	0·722	0·642	0·671
Significant difference (log. scale $P = 0·05$) = 0·030			

IV. *The Seasonal Growth of Bluebell Plants subjected in the Previous Year to Different Levels of Light Intensity and Mineral Nutrient Supply*

So far, in this series of investigations, attention has been confined to the direct effects of varying light intensity and nutrient supply on the growth of *S. non-scripta* and there yet remains for consideration whether there may be delayed effects of such treatments which only show themselves in the subsequent season. To investigate these possible after effects, mature dormant bulbs were taken from two experiments (for further details see Blackman and Rutter, 1947, expt. III, 1938, and expt. IV, 1939) in which there were 24 treatments made up of 3 light intensities and 8 levels of mineral nutrient supply, namely, C, N, P, K, NP, NK, PK, NPK. Bulbs from each of the 24 treatments of light intensity and nutrient supply level, after being lifted

and weighed in the summer, were replanted in the autumn of the same year and their seasonal growth under the conditions of full daylight was measured in the following spring.

In expt. XX, 15 weighed bulbs, taken from each of the 24 treatments of expt. III, were grown in 1939. Each lot of 15 was divided into 3 replicates of 5 bulbs each, and by confounding the interaction between nitrogen, phosphorus, and potassium with block difference, the 72 plots were planted in

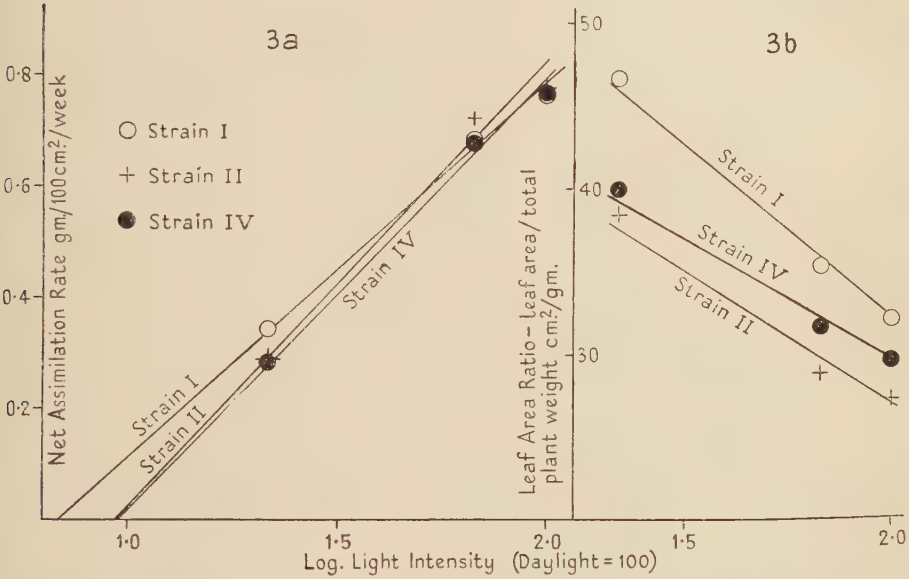

FIG. 3. Experiment V. The effects of varying light intensity on (*a*) the net assimilation rate, and (*b*) the leaf-area ratio of three strains of *Scilla non-scripta*: the observed means and fitted regressions are both given.

6 blocks. The 24 combinations in each replicate will be referred to as pre-treatments.

There was inevitably a considerable variation in initial bulb size, due to the changes in bulb size produced by the previous light treatments. In consequence, in order to test the significance of the pre-treatment effects, it was necessary to carry out an analysis of covariance, correcting the growth made on to a standard initial weight. After correction the difference between the initial weight and the weight at the time of sampling has been selected on statistical grounds as a measure of growth, since this quantity is statistically independent of initial weights. In order to produce a more normal distribution of the variables, a logarithmic transformation has been employed.

The plots were first sampled on May 16 when 3 plants were collected, while the remaining 2 plants—or rather bulbs—were dug up on August 2, when the leaves had died back and growth had ceased. After correcting for differences in initial bulb size, the statistical analysis showed that there was a significant

effect of pre-treatment at the first sampling occasion, in that plants which had been subjected to a low light intensity in the previous year had grown faster than those exposed to full daylight. However, a similar analysis of the data from the sample of August 2 showed no such significant effects.

The changes in weight relative to the mean initial weight of one sample are shown in Fig. 4 for the two sampling occasions. From these results it is concluded that, while the growth made during the whole season is unaffected

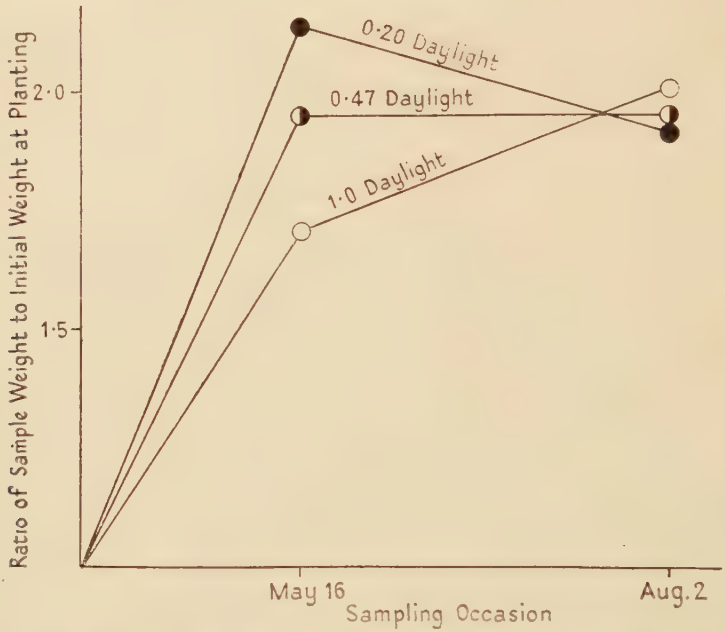

Fig. 4. Experiment XX. The effects of varying light intensity in one season on the growth of *Scilla non-scripta* in the following season when all plants received full daylight. The ratios of sample weight in the second season to initial weight in the previous autumn have been corrected for differences in bulb size.

by the light intensity pre-treatment, a greater proportion of the growth is made early in the season by the plants which had been most heavily shaded in the previous year. Thus, on May 10, plants receiving 0·2 daylight in 1938 had reached their maximum dry weight, whereas those from the full daylight pre-treatment had yet to make a considerable amount of growth.

In seeking for an explanation of these effects it seemed possible that the earlier and faster growth of the pre-shaded plants might be due to the earlier emergence of the leaves and a larger leaf area. The proportion of leaf weight to total weight was therefore calculated for each plot from the data of the first sampling occasion. However, the means of the plants subjected to the three light pre-treatments of 1·00, 0·47, and 0·20 daylight were very similar, namely 0·141, 0·140, and 0·142 respectively. Unless it is postulated that the relation of leaf area to leaf weight had been altered by the pre-treatment, the differ-

ences in growth rate cannot readily be accounted for in terms of increased leafiness.

In 1940 a similar experiment (expt. XXI) was carried out with bulbs drawn from expt. IV, 1939. Samples were taken on May 3, when the leaves had reached maximum development, and again on June 4. Measurements of leaf area were made on each date so that any differences in growth rate could be analysed in terms of net assimilation rate and relative leaf area.

When the results from the June 4 sample are corrected for differences in initial weight, the analysis of covariance reveals that there are no significant differences between the three light pre-treatments. The mean weights at this sampling occasion corrected on to the mean initial weight are shown in Table XIV.

TABLE XIV

Experiment XXI, 1940. The Residual Effects of Variation in Light Intensity in One Season on Growth in the Subsequent Season

Dry weight of plant (g.)			
October 1939.	June 4, 1940.		
	Intensity of light pre-treatment (full daylight = 1·00).		
	1·00	0·59	0·22
1·507	3·099	3·587	3·323

Thus if there had been an effect similar to that in the preceding year, i.e. a higher growth rate at the beginning of the season, this sample was taken too late to show it. The earlier sample of May 3 is not suitable for comparison with the initial weight of the previous autumn because over this period there had been a net loss in weight due in part to respiration losses during the winter (Blackman and Rutter, 1947) and the sloughing off of the depleted bulb scales in the spring at the time of leaf emergence. However, an analysis of covariance applied to the increase in weight over the period May 3 to June 4 also revealed no significant effect of light pre-treatment.

In neither of these experiments was there any evidence that differences in the levels of nutrient supply in the previous year significantly affected the growth rate in the subsequent season.

The data of expts. XX and XXI also provide information on the general relationship between rate of growth and bulb size. If x is the initial weight and y the increase in weight, then it may be assumed that the regression relating to y and x passes through the origin if the relationship is linear, but will not do so if the relationship is curved. But if when a linear regression of the form $Y = a+bx$ is fitted to the data, a does not differ significantly from 0, the relation between y and x will also be linear.

In order to attain more normal distributions, the analyses of covariance have been carried out on $\log y$ and $\log x$, so the test of linearity is not whether $a = 0$ in $Y = a+bx$, but whether $b_1 = 1$ in $\log Y = a_1+b_1 \log x$.

The regressions for the two sampling occasions of May 16 and August 2 of expt. XX and the June 4 sampling date of expt. XXI are set out below:

Experiment XX

May 16: $\log Y = 0{\cdot}040 + (0{\cdot}834 \pm 0{\cdot}109)\log x$ (i)

Aug. 2: $\log Y = 0{\cdot}222 + (0{\cdot}543 \pm 0{\cdot}123)\log x$ (ii)

Experiment XXI

June 4: $\log Y = 0{\cdot}302 + (0{\cdot}813 \pm 0{\cdot}102)\log x$ (iii)

Only in the case of equation (ii) does the coefficient of log x differ significantly from 1. Hence it is concluded that, over the period from the autumn until early June, the dry weight increase is linearly related to the initial bulb weight. But when the whole of the annual growth cycle is considered, the growth increment relative to initial weight becomes less as bulb size increases.

Two alternative explanations can be put forward to account for these differences. Firstly, that the ratio of inflorescence size and seed production to total plant weight are higher in large bulbs, so that in the ripening phase, proportionately less material is transferred to the bulb. Secondly, that the leaves of the smaller bulbs persist for longer periods.

Discussion

This is the last paper in this series which will be especially concerned with the autecology of *Scilla non-scripta*, and a discussion of the factors which determine the distribution in different communities must inevitably demand frequent reference to the earlier papers. For the purposes of clarity, the references may cover more detail than normal. In addition, it is proposed to include in the discussion the results of some experiments or observations which have not been previously described, either because they did not conveniently fall into the various sections or are not extensive enough to be considered as separate sections.

Taking first the factors which limit the distribution of the bluebell in grassland communities, it seems clear that the dominant species of grasses in the sward, the factors which control their rate of growth in the spring, and incidence and the intensity of grazing and trampling are all concerned. On the evidence of expts. XI–XVIII it is concluded that the bluebell cannot be a component of the sward in communities which carry a high density of grazing animals from early in the spring. On the basis of expts. XIV–XVIII it is not, however, possible to separate precisely the relative importance of defoliation and trampling. To carry out a precise assessment of defoliation alone would involve many complex and costly experiments to test how far the leaves of the bluebell relative to those of different grasses were palatable or unpalatable to different types of grazing animal. It has, however, been observed that bluebells planted experimentally in woodland away from other vegetation may be heavily grazed by rabbits, but this does not imply that in a mixed sward there would not be a preference by rabbits for other species: indeed, observation suggests that this is so.

With a high density of grazing animals, such as sheep or cattle, the intensity of trampling will still be a major factor even if little defoliation of *S. non-scripta* takes place. The period and duration of the trampling must, however, be taken into account since it is apparent from expts. XVII and XVIII (Tables X and XI) that a combination of frequent and early trampling in the spring is the most injurious.

These biotic factors will in turn be dependent on the type of grassland community, the type of grazing animal, and the management of grazing by man. It has been shown that the growth of grassland in the spring is controlled by rising temperature, nitrogen supply, and the dominant species in the sward (Blackman, 1936; Jones, 1933*a*, 1933*b*). Growth commences earliest where there is a high available nitrogen status in the soil and the dominant species consists of early grasses, such as *Lolium perenne*. In fact, in southern England, stock will be turned into such grassland at a time when the bluebell is just beginning to make active growth in April (see Blackman and Rutter, 1947). Thus the liability to injury from trampling will be maximal under such conditions.

On the other hand, the spring growth of the sward will be delayed if there is a lack of available nitrogen in the soil and the dominant species consist of the types which start growth later in the season because they demand a higher level of temperature for active growth, e.g. *Agrostis* spp., *Holcus* spp., *Festuca* spp. In consequence, if stock are not permitted to graze the sward until considerable growth has been made, then under such conditions of a late spring growth delay in the incidence of trampling will favour *S. non-scripta*.

There are, however, major differences in the control of the grazing exercised in these early or late types of grassland. In the latter, the control of grazing is minimal and often is little different from the free-range grazing. Here the density of stock is limited by the food available during the winter months, so that the number of animals per unit area is not high. Moreover, as soon as active growth starts in the spring, the grassland will be understocked, there will be less movement of the animals in search of food, and the intensity of trampling will be slight. There will, however, be a critical period in the early spring between the emergence of the bluebell leaves and the rapid growth of the grasses when the sward will be grazed hard and more heavily trampled.

Coupled with the effects of trampling and defoliation, there is the direct competition between *S. non-scripta* and the grasses. From expts. XI–XIII (Tables I–V) it is clear that the competitive conditions which are most inimical to the growth of the bluebell are those in which the grasses are not grazed and have received additional nitrogen. In contrast, on the evidence of expt. XI (Table I) the most favourable conditions will occur where there is a high level of nitrogen supply and only the grasses are grazed without any attendant heavy trampling. Conceivably, such selective grazing could occur with rabbits, but would certainly not result from grazing by larger herbivores. Again, a high availability of nitrogen in the spring implies a fertile soil and these conditions

in turn will favour the dominance of early grasses like *L. perenne*. In consequence, such grassland will be the first to be grazed in the spring by stock—a set of conditions which will be wholly inimical to the bluebell. Conversely, if the sward is not grazed, it is apparent from Table I that the adverse indirect effect of a high nitrogen level in increasing the growth and thereby the shade cast by the grasses more than outweighs the advantages of increased nitrogen supply to *S. non-scripta*.

If shading is a major factor in the competition between the bluebell and grasses, then it would be expected that seedlings or very small plants of *S. non-scripta* would be more adversely affected than larger plants, and this is supported by the results of expts. XII and XIII. It would also be expected that the degree of shading would be greater in the case of tall grasses, provided that no other major factor is involved. The fact that the smallest bluebell plants growing in competition with *F. rubra* made less seasonal growth than those competing with the taller *D. glomerata* (see Tables IV and V) suggests that other factors may also be operative.

There is also evidence that the degree of competition varies with the season. In the case of the comparable experiments for 1939 and 1940, where *F. rubra* was in competition with the smallest plants, in 1939 the seasonal gain in weight was 99 per cent. in the uncut sward receiving nitrogen and 132 per cent. on the control, while for the comparable treatments in 1940 the plants lost weight (Table V). It is considered that this seasonal variation is due to the differences in spring rainfall; 1940 was a particularly dry year and 1939 a wet one. That in 1940 water availability was a more important factor in limiting growth than in the previous years is confirmed by the experiments on light intensity and mineral nutrient supply (Blackman and Rutter, 1947, expts. III, IV, V, VII, VIII).

In considering therefore the interrelationship between the biotic factors and those concerned with direct competition with the grasses, it appears that the following set of conditions would be most favourable to *S. non-scripta*: (i) the soils should be of a low mineral nutrient status; (ii) the dominant grasses should possess a prostrate habit; (iii) the grasses should not start active growth until late in spring; and (iv) the sward must not be grazed until after the bluebell plants have flowered. The fact that *S. non-scripta* is more frequently found outside woodland in Scotland, in Wales, and in the south-west and north of England is therefore significant. It is in these regions that the soils tend to be leached. It is here that grassland communities dominated by such grasses as *Agrostis*, *Festuca*, and *Holcus* species are of more frequent occurrence. It is here that there is a higher proportion of rough or hill grazings which in turn provide sites where there is either no grazing at all because of the steepness of the slopes or where, at the higher altitudes, grazing in the early spring does not take place because the flocks have not been moved up from wintering in the valleys.

Although it is clear that these favourable conditions are found less frequently in the south and east of England, it must be emphasized that there

is a complicating factor, namely, that in the west and north of England there is a higher rainfall. In fact, for the drier areas there is some data which suggests that even with the most favourable combination of the factors already defined, the conditions in grassland are rarely advantageous to *S. non-scripta*.

This evidence is forthcoming from an experiment in which bulbs of flowering size were planted in an infertile gravel soil near Slough where the vegetation had not been grazed for several years, but had from time to time been cut in the summer. In consequence, the dominant grasses consisted of *F. ovina* and *Arrhenatherum elatius*, together with *Hypericum perforatum*, *Centaurea nigra*, and *Lathyrus pratensis*. After the dominant bulbs were planted in the autumn of 1937, they were left for 4 years and dug up again in July 1941, when they were in the dormant phase. During this period the following series of treatments were applied. In one series the herbage on the plots was left to grow without any interference throughout the year, but some of the plots received each year in the early spring either additional nitrogen (50 lb. N per acre) or phosphorus (75 lb. P per acre) or potassium (75 lb. K per acre). In another series the plots were defoliated once either before the flowering of the bluebell or at a time when the plants were in flower. In the third series the plots were *permanently* covered with screens either of butter-muslin or perforated zinc for the whole of the four years.

TABLE XV

Experiment XXII. The Effects of Additional Nutrients, Defoliation, and Shading over a Period of Four Years on the Growth of S. non-scripta *in a* Festuca-Arrhenatherum *Sward*

Treatments.	Final weight (log. wt. of 10 bulbs).	Ratio of final to initial weight.
Series I. No defoliation of sward		
(i) Control	0·965	0·87
(ii) Nitrogen added to sward . .	0·974	0·91
(iii) Phosphorus added to sward. .	0·964	0·89
(iv) Potassium added to sward . .	0·963	0·90
Series II. Sward defoliated		
(i) Before flowering of bluebell . .	0·749	0·57
(ii) When bluebell is in flower . .	0·878	0·69
Series III. Sward shaded		
Intensity of shade 0·5–0·6 daylight .	1·289	1·64
„ „ „ 0·2 „ .	1·199	1·80
Sig. diff. between treatments ($P = 0·05$)	0·129	

From Table XV it is evident that in the ungrazed sward, irrespective of the level of nutrient supply, there has been over the 4 years a decrease in weight of approximately 10 per cent. Moreover, the reduction has been increased to 30 per cent. even when a single defoliation has taken place as late as the full flowering stage. Since these results have been obtained with flowering plants and in view of the evidence that small plants are less competitive than

large plants, it is concluded that at least in the drier areas, *S. non-scripta* will seldom invade grassland communities through seed dispersal, but that mature plants may survive for long periods where woodland has been cleared and there is no or very little grazing in the spring.

Perhaps the most interesting results in Table XV are those for Series III, where shading has clearly shifted the balance of competition in favour of *S. non-scripta*. This shift could be explained on the basis that the bluebell is better adapted to conditions of low light intensity than the other components of the community. Before, however, considering the available evidence for such a conclusion it must be emphasized that the figures given for the degree of shading, namely 0·5–0·6 and 0·2 of daylight, are somewhat misleading. During the spring the percentage of light transmitted by the screens were as stated, but later in the season the figures are too high. In order to minimize the proportion of lateral light reaching the plants under the screens they were placed in position some 12 in. above the ground. As a result, by midsummer, the tops of the shoots of the grasses and other species reached and pressed against the surface of the screens, so that the lower halves of the plants were much more heavily shaded. Moreover, the effect was greatest in the first year, since the initial growth made in the shade will in part be due to the utilization of reserves in the leaf bases and roots (Blackman and Templeman, 1938).

The experimental shading therefore had the effect of increasing the degree of shading in the summer months below 0·5 and 0·2 daylight. Thus the conditions were such that the shading was more intense for the grasses and other plants whose seasonal growth extended from the spring to the autumn than it was for *S. non-scripta* with a growth cycle confined to the spring months. In other words, the light conditions were not unlike those of deciduous woodland.

As to whether the light requirements of *Festuca* and *Arrhenatherum* and the other species are greater than those of the bluebell, there is no direct evidence. There is, however, indirect evidence from the results of investigations, which will shortly be published in this series, that in comparison with some ten other species of plants the reactions of the bluebell to low light intensities are by no means exceptional.

Turning next to a consideration of woodland conditions, all the evidence of the previous series of investigations emphasizes that the environment is favourable to *S. non-scripta*, not because of the shade but because in woodland heavy grazing animals are absent and therefore adverse biotic factors do not operate.

However, in woods which are much frequented by man the trampling factor is not entirely absent. For example, in the woodland investigations it was observed that even in a single season the paths taken to the experimental areas could be picked out by the bruised leaves. Such trampling will certainly occur where many people come to pick the flowers, and it is this trampling rather than the picking which will be injurious. There can be no possibility that the actual plucking of the flower-stalk is damaging to the bud initials, since

periodic dissection of samples of the bulbs has shown that the primordia of next year's leaves and inflorescence have not been formed.

In a recent paper Peace and Gilmour (1949) have reached the same conclusions that neither picking the flowers nor pulling the flower-stalk has any deleterious effect on the number of flowers produced in subsequent seasons. They too concluded that the trampling and bruising of the leaves associated with picking could lead to a considerable reduction of flowering.

It is of some interest to note that in one of their woodland experiments there was some indication that removal of the inflorescence increased the number of flowers in the following years. Such an increase would be expected on the basis of the previous investigations (Blackman and Rutter, 1947) since there will no longer be a partition of the assimilates between the developing inflorescence and the bulb, and this will lead to larger bulbs and larger bulbs will in turn tend to split and produce offsets and hence more inflorescences in the following seasons.

In fact, under conditions which favour vegetative reproduction rather than dispersal by seed, picking the flowers, as long as the effects of trampling are minimal, may be advantageous. The removal of the inflorescences is not confined to man's activities, for it has been observed on several occasions in woodland that the flower-stalks have been bitten off (probably by rabbits) at the time of flowering.

In the majority of woods the biotic factors so far instanced can be regarded as unimportant, but the possibility that the distribution of the bluebell may be related to the amount of fallen leaves has yet to be considered. It is self-evident that the annual deposition of leaf litter must bear a relationship to the denseness of the overhead canopy and this in turn will be related to the degree of shading. The direct relationship established in the woodland investigations between bluebell density and the degree of shading might therefore be interpreted in terms of variations of litter accumulation. The evidence of the woodland investigations (Blackman and Rutter, 1946) does not support this view. In two of the woods, a *Larix japonica* plantation and a beechwood, the marked gradient in light intensity on the experimental areas was due rather to 'side light' entering from the edge of the wood than to variations in the overhead shade. In consequence the correlation between the degree of shading and leaf deposition was small. Again, in the third wood, where the density of the bluebells under holly-trees and a canopy of mixed ash and beech was related to variations in light intensity, statistical analysis did not indicate that the differences in litter deposition or the type of litter were significant factors.

The possibility that the type of litter and rate of breakdown might affect the supply of nutrients has already been discussed in the 1947 and 1949 papers, and it was concluded that for established plants light rather than nutrient supply is the controlling factor in closed woodland. On the other hand, where there is a slow rate of breakdown and a dry surface layer of undecomposed leaves, the conditions are less favourable for the establishment of seedlings.

In considering the pre-eminence of the light factor and the distribution of *S. non-scripta* in these woodland communities, it should be emphasized that where other plants were present in the ground flora their density was not great and they were of a prostrate habit and did not compete for light. Such conditions do not always hold; for example, where *Mercurialis perennis* is also present, *S. non-scripta* is generally not found if the density of the shoots of dog's mercury is high and their growth is luxuriant.

In open woodland, where the level of light intensity during the summer months is higher than in closed woodland and plants other than those with a spring growth cycle can therefore grow successfully, competition between the bluebell and other components of the ground flora may be accentuated. For example, grasses such as *Holcus mollis* may occur, while taller plants such as *Rubus* spp. and *Pteridium aquilinum* will also be present.

On the basis of the present series of investigations it is of some interest to re-examine the conclusions reached by Woodhead (1904, 1906) on the inter-relationship between *S. non-scripta*, *H. mollis*, and *P. aquilinum* in some Pennine oak woods. In the deepest shade the bluebell is found growing alone, in areas of intermediate shade all three plants occur together, while in the most open sites where *P. aquilinum* makes luxuriant growth it tends to become dominant. It is not considered that the presence of the bluebell in the areas of lowest light intensity can be ascribed to its greater ability to withstand shade, for it has already been shown that the compensation point for *S. non-scripta* is approximately 0·09 of daylight (Blackman and Rutter, 1948), while Salisbury (1918) concluded that bracken was not found in the oak hornbeam woods where the light intensity fell below 0·04 of daylight in midsummer and did not form a closed society below a minimum intensity of 0·11 daylight. From the previous studies of the seasonal fall in light intensity of different woodlands (Blackman and Rutter, 1946) it is evident that a light intensity as high as 0·11 daylight in midsummer is only found in open woodland. Secondly, considering the lower limit for bracken of 0·04 daylight, this mid-season intensity will correspond to light intensities in the early spring of 0·6 daylight or more, according to the type of woodland.

The contrast does not therefore lie in the varying reactions to deep shade of the two species but in the difference between the species as to the time of the emergence of the shoots and commencement of active assimilation. The bluebell will have made approximately half or a third of its seasonal growth by the time leaf expansion of the oak-trees is taking place, while the expansion of the bracken fronds will not be complete before the oak canopy has fully developed. Where *S. non-scripta* is growing alone in closed woodland, that is where the *minimum* mean spring light intensity is of the order of 0·05 daylight, the total seasonal growth made will be compounded of the gain made in the high-light phase, and the loss during the low-light phase when the degree of shade will be below the compensation point. Where bracken is also present, i.e. in the less shaded areas, though there will be some advantage to *S. non-scripta* in the increased light intensity during the high-light phase, this

will be offset later by the deeper shade in the low-light phase. Then the shading will in part be due to the canopy and in part due to the bracken, and the greater the height and density of the fronds, the lower will be the light intensity. It is considered that it is this direct shading by dense bracken which is operative in the suppression of *S. non-scripta*.

In the case of bluebell seedlings, apart from the light factor, it is probable that where the accumulation of bracken litter is pronounced, the conditions are unfavourable for establishment.

Turning next to the association between *S. non-scripta* and *H. mollis*, the essential criteria for the successful establishment of bluebells in grassland will also hold. There will be no grazing, *S. non-scripta* will start growth well in advance of *H. mollis*, and the rainfall in the Pennines is relatively high. The main difference between the woodland and grassland environments will be that at all times in woodland there will be some overhead shade, but the degree of shading will be greater for *H. mollis* than for *S. non-scripta* since with its longer and later period of growth the grass will be more subject to shading by the bracken and the trees. In fact, the conditions will not be unlike those of expt. XXII, where the grassland plots were permanently shaded and 'self-shading' became progressively greater as the season advanced. From the aspect of the bluebell, the tree shade during the high-light phase will restrict growth more than in grassland. This disadvantage will, however, be offset by the effects of mid-season shade in decreasing the growth of *Holcus*, so that there will be less active growth in the spring and therefore less competition with the bluebell.

In many of the damper oak woods it has been observed that where the canopy is not continuous or the shrub layer is not well developed, *Rubus* spp. rather than *P. aquilinum* are often dominant and in such areas *S. non-scripta* is absent. This pattern of distribution, it is considered, can again be interpreted in terms of the light factor. Even during the high-light phase, the bramble shoots cause a marked reduction in light intensity at ground level and this is further accentuated when new foliage is produced. Such conditions are akin to those in evergreen woodland where, with the absence of an initial high-light phase, the mean degree of shade is in general inimical to the bluebell (Blackman and Rutter, 1946).

Although the vernal fall in light intensity in deciduous woodland contrasts most with the small decrease in evergreen woodland, there is experimental evidence that the mean light intensity during the period of active growth is more important than the overall fall in intensity between the high- and the low-light phase. For example, at the Field Station, Slough, dormant bulbs of known weight were planted in groups of 10 in a number of sites so selected that the shade was provided either by evergreen plants (e.g. *Pinus sylvestris*, *Viburnum tinus*) or by deciduous trees (e.g. *Betula alba*, *Corylus avellana*, *Sambucus nigra*). Planting took place in the autumn of 1937, and in the following spring the degree of shade was measured at weekly intervals for each site until June, when the bulbs were lifted and reweighed.

From Fig. 5 it is seen that the change in weight, expressed as the ratio of the final to the initial weight in the previous autumn, largely follows a logarithmic relationship with mean light intensity, irrespective of whether the fluctuations of light intensity about the mean are large (deciduous overhead canopy) or small (evergreen canopy). It should also be noted that below 0·09 daylight there is a net loss in weight and that at the light intensities of 0·05 and 0·014 of

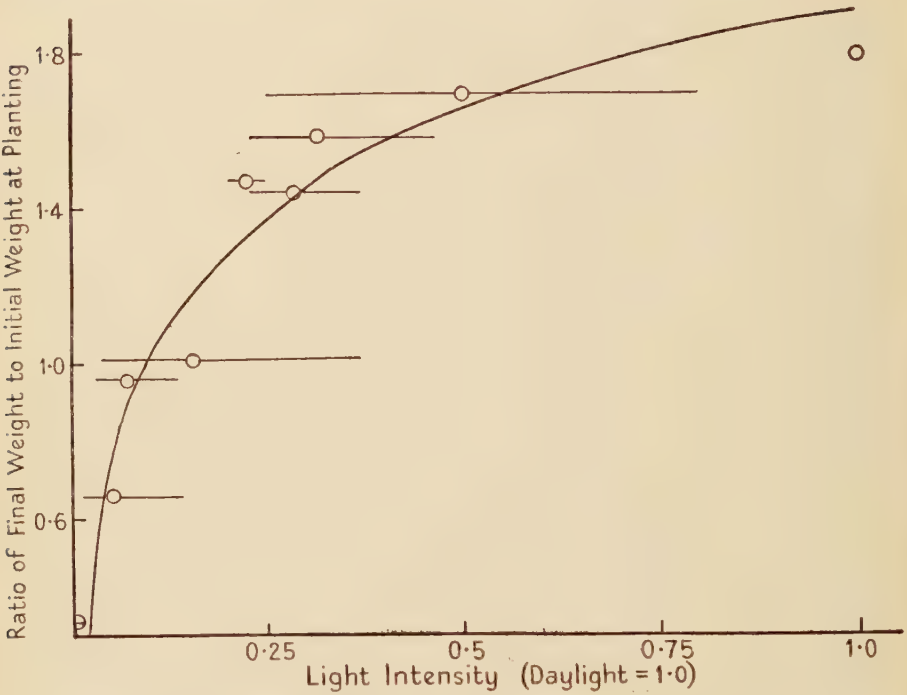

Fig. 5. Experiment XXIII. The variation in the seasonal growth of *Scilla non-scripta* subjected to shading by different trees and shrubs. Besides the mean light values during the spring (end of March to June) for the nine experimental sites, the minimum and maximum degree of shading has also been indicated for each site.

daylight the losses over the yearly cycle amount to 34 and 66 per cent. respectively. It is therefore clear that even plants with large bulbs cannot persist for long in deep shade.

Although it is evident from Fig. 5 that over the wide range of light intensity from 1·0 to 0·01 of daylight the mean light intensity rather than the fluctuations about the mean control the seasonal growth, it does not follow that within a narrow range this is also true. It has been shown previously (Blackman and Rutter, 1948) that where plants have been grown in full daylight until the beginning of May and have been subsequently shaded they make less growth than plants that have been constantly shaded at a comparable mean light intensity for the whole period. It was also demonstrated that this difference was due to the greater assimilating area of the constantly shaded plants.

This aspect of the light factor must be taken into account in considering the association of *S. non-scripta* with bracken in sites where there is no overhead canopy. In the high-light phase before the expansion of the bracken fronds, the net assimilation rate will be higher than in the woodland sites, but the leaf area will be less. The differences therefore in the growth made within the two light environments will be dependent on the balance between assimilation and leaf area and these, in turn, will be dependent on the differences both in the length of the high- and low-light phases and in the relative light intensities operating over the two periods.

In deciduous woodland under conditions which permit of a close stand of bracken, the highest light intensity will be of the order of half to three-quarters of daylight, but the intensity will fall rapidly as the tree leaves expand and this will normally take place before the expansion of the bracken fronds is complete. On the other hand, in the open, once the bluebell leaves have emerged through the litter, they will be receiving full daylight and the high-light phase will persist for longer than in woodland. However, in the low-light phase, especially where there is a very vigorous growth of bracken in the open situation, the degree of shading will be of the same order under both sets of conditions, i.e. generally below 0·05 of daylight.

On the basis of these considerations and the results of the earlier investigations, it is concluded that in the open sites the effects of length and intensity of the high-light phase in raising the net assimilation rate will more than counterbalance the larger leaf area of plants growing in woodland. In consequence, the seasonal growth will be greater. From general observation of sites outside woodland it is clear that the bluebell will persist in tall and dense stands of bracken. For example, in Bagley Wood, near Oxford, *S. non-scripta* can still be found in an ungrazed area which was cleared of trees over 30 years ago and where the bracken now reaches a height of 2 metres.

This equilibrium between the bluebell and dense bracken is not only related to the light factor. Because of the deep shade cast by a dense stand of *P. aquilinum*, plants such as the grasses, which have an extended growing season, are suppressed and competition between the bluebell and other plants during the spring will be restricted to those which also make their active growth in the high-light phase. Such a limitation of competing species may well be more important in areas of low rainfall.

The establishment of dense stands of bracken implies that at least during the summer months heavy grazing animals have not been present in any numbers on the area, since the consequent trampling will be inimical to the formation of such closed communities. The association of bracken and bluebells found on steep slopes in the wetter parts of Great Britain will therefore in part be dependent upon their inaccessibility to grazing animals. On the other hand, slopes in less humid areas, where the soil dries out rapidly, will be unfavourable habitats for *S. non-scripta*.

In considering the occurrence of bluebells on slopes in areas of high rainfall another factor must be taken into account. Under such conditions there will

be free drainage and no possibility of a temporary waterlogging of the soil. Although general observation indicates that *S. non-scripta* will grow under relatively wet conditions, there is evidence that it will not tolerate waterlogged soils.

For example, in the Japanese larch plantations at Shurlock Row, Berks., where the relation between bluebell density and light intensity was investigated (Blackman and Rutter, 1946), it was observed that in one area of relatively light shade bluebells were singularly absent except on the raised banks of a ditch. In order to investigate experimentally the growth of bluebells in this area, 60 graded dormant bulbs of known weight were planted in the autumn along transects running inwards at right angles to the edge of the plantation. During the latter half of the subsequent winter this area was waterlogged and a high proportion of the planted bulbs failed to emerge above ground in the spring. On investigation it was found that this failure was due to the rotting of the bulbs by *Penicillium cyclopeum*. That the attack of this fungus is favoured by a high soil moisture content has also been found by Singh (1941). It is not, however, inferred that attack by this mould is confined to such conditions, since during the collection of dormant bulbs from several woods it has been observed that a number of the bulbs possessed necrotic lesions. On the other hand, in other sites when bulbs were dug up during the spring at periodic intervals, serious damage has not been noted.

Lastly, in considering the main factors responsible for the distribution of *S. non-scripta* there remains the question of the importance of soil types, and here it is not so much a matter of enumerating the most favourable soils but of defining the soil conditions where the bluebell is absent. Watt (1934) points out that on the Chiltern plateau beech woods *S. non-scripta* may be abundant on patches of sand, but is rare or absent on adjacent areas of clay with flints. In beech woods of the Berkshire Downs it has been observed that where the chalk is very close to the surface the bluebell disappears. Again, well-defined podzols are in general unfavourable habitats, but bluebells will grow under less acid conditions, i.e. soils showing weak podzolization but with a mull surface layer.

The experimental results of the 1947, 1948, and 1949 papers in no way indicate that variations in the nutrient status of different soils are important. On an infertile gravel soil where the growth rate of other plants was greatly increased by the additions of nitrogen, phosphorus, and potassium, the gains made by the bluebell were small and in an oak wood negligible. Again, since *S. non-scripta* even in full daylight only doubles or trebles its weight during the year, it is unlikely that there can be a demand for a high nutrient level in the soil, and in woodland with the consequent slower rate of annual increase the need will be even less.

Since it is on the lighter soils, in contrast to some heavy clays, that the bluebell is prevalent, the difference may be in the physical characteristics. As Chouard (1926) has observed, bluebell seedlings at an early stage start to form a bulb and produce contractile roots, and with the years there is a progressive

movement downwards to a maximum depth depending on the soil type. Since a bluebell seed only weighs 0·005 g. and since at 0·2 daylight bulbs will only double their weight in the season, this process is inevitably slow. It is estimated that in woodland by the time bulbs reach their maximum depth they will be at least 7 to 10 years old.

Therefore during the period of establishment the plant will remain in the surface layers, which in dry areas or dry springs will be most prone to dry out. If clay soils prevent or slow down the downward movement of the bulb, then the liability to drought may be increased. Such an explanation could be equally applicable to very shallow calcareous soils where the underlying chalk would offer a physical barrier to further downward movement.

As an alternative explanation it could be put forward that the depth to which bulbs penetrate is determined by the carbon dioxide or oxygen tension of the soil atmosphere, and that in clay or very wet soils a steep gradient with depth of oxygen or carbon dioxide concentration, or a combination of both, may confine the bulbs to the surface layers. Certainly, in light soils, the larger bulbs may reach a depth of 20–25 cm., while at the other end of the scale in wet situations the limit may be 10 cm. or less. Such a variation has been observed on a gentle slope terminating at the bottom in marshy ground.

There is perhaps one further possible environmental factor which must be taken into account in assessing the differences within and without woodland habitats. The overhead canopy will give some protection from the effects of spring frosts and wind velocities will be lower than in the open. The available evidence does not, however, indicate that the shelter provided by woodland is important. In the three years when bluebells were planted in the open, no signs of frost injury were observed and the plants made more growth than those which were lightly shaded and therefore to some extent protected. Again, it has been already stated in the introduction that *S. non-scripta* can be found growing on rock ledges and the tops of cliffs exposed to the Atlantic. It is, however, possible that in areas of low rainfall high winds may have an indirect effect by increasing evaporation of water from the surface soil.

Lastly, there remains for discussion the underlying causes for the differences in the growth rates of the five strains of bluebell. From expt. XX there is evidence that variations in the light intensity in one season may affect growth in the subsequent year, and it will be recalled that whereas bulbs of four out of the five strains were collected from woodland sites, the fifth (strain IV) had been grown in the open. In consequence, the 'light histories' in the year previous to expts. V and XIX were very different. Since the soil conditions also differed between sites, it might be advanced that differences in the mineral nutrient reserves between strains might carry over to the subsequent year. Again, in expts. XX and XXI it has been established that the rate of seasonal growth is dependent upon bulb size and, as the initial bulb weights varied between strains, this variable must also be taken into account.

Considering first the hypothesis that the differences between strains are related to variations in the mineral nutrient reserves, the available evidence

is against such an assumption. In expts. XX and XXI variations in the level of nitrogen, phosphorus, and potassium in one season in no way influenced growth in the following year. Moreover, in the 1947 and 1949 papers it has been shown that below half-daylight the level of nutrient supply has little effect on the growth rate, while it is clear from Figs. 1 and 2 that the differences between strains persist at 0·22 and 0·11 of daylight.

Taking next the question of how far variations in light intensity between habitats may have influenced the growth rate in the subsequent experimental period, it is considered that the differences between strains are far too large to be accounted for on this basis. For example, in expts. XX and XXI there were no significant differences in the adjusted ratio between the final and initial weights of plants which had been grown under varying light intensities (1·0, 0·59, and 0·22 daylight) in the previous year.

Turning next to how far differences in initial bulb size between strains may have affected the differences in seasonal growth, in several comparisons this question does not arise since the initial bulb weights were very similar. In expt. V, strains I and IV had the same initial weight but yet differed in their growth rates. The results of expt. XIX require more consideration since the initial bulb weights of the five strains ranged between 0·566 and 0·701 g. It is, however, possible to gain some idea from expt. XX of the magnitude of the variations in seasonal growth in terms of the variations in initial bulb size. For plants growing in full daylight it is possible to calculate by means of formula given on page 504 the ratio between final and initial weight; over the limits of 0·55 to 0·79 g. the ratio varies from 1·69 to 1·76 for plants given full daylight. On the other hand, within precisely the same limits of initial bulb size, the variations in the ratios for the five strains are from 1·98 (strain IV) to 3·65 (strain V). In view of these large differences it must be concluded that the differences in the growth rate between strains are inherent characteristics.

The further analysis of the differences between strains I, II, and IV in expt. V (Fig. 3) has shown that the variations in growth rate are independent of the net assimilation rates but dependent both on the relative leaf-area ratios and the time of shoot emergence in the spring (see Table XIII). The importance of the high-light phase in the seasonal growth of the bluebell in different environments needs no further emphasis, and it is clear that an ecotype which produces a large leaf area early in the spring has a potential capacity for successful establishment under a wider range of habitats. On the other hand, it does not appear that ecotypes vary in their ability to survive in deep shade.

In conclusion, it is hoped that this discussion has served to emphasize how great are the advantages of linking general ecological observations with both precise field studies and multifactorial experiments for a proper understanding of the interaction and relative importance of environmental factors. In the case of the bluebell a proper understanding has led to a new interpretation of the factors which limit its distribution both within and without woodland. Further investigations are now required to determine whether this inter-

pretation is also applicable to other woodland plants, especially those species which make active growth in the high-light phase of spring. In view of the fact that several woodland plants can be found growing in the open at high altitudes, it may well be that they too grow best in full daylight and are normally prevalent in woodland because they are there protected from trampling and grazing by herbivores.

There is also a great need for further precise investigations on the growth and development of plants in different habitats. For this purpose the techniques of growth analysis will serve as a valuable link between the field and the laboratory: indeed, on occasion, they may also serve as a useful corrective. The concept of an obligate shade plant rests largely on laboratory investigations in which the assimilation of leaves rather than whole plants has been measured at varying light intensities, and the conclusion drawn that the assimilation rate may reach a maximum at light intensities far below full daylight. The present field investigations have demonstrated that up to full daylight the net assimilation rate of *S. non-scripta* is logarithmically proportional to the light intensity. There is supporting evidence that this relationship also holds for *Geum urbanum* and *Solanum dulcamara*. In fact it will be shown in a later paper that variations in the growth rates of plants under shade conditions are related to differences in the total leaf area, rather than differences in the net assimilation rate.

Summary

In the four previous papers it has already been established, firstly, that *Scilla non-scripta* grows best in full daylight, secondly, that the light intensities ruling in closed deciduous woods are the major factors controlling the growth rate and distribution, and thirdly, that the bluebell does not require a high level of mineral nutrients. Yet, on the other hand, *S. non-scripta* is normally confined to woodland and it is only in restricted localities that this plant occurs outside woodland, more particularly in grassland or bracken (*Pteridium aquilinum*) communities. The present paper is concerned with the further analysis of the factors which may operate in limiting the distribution.

When the bluebells are planted in swards dominated by one of the following grasses—*Phleum pratense*, *Festuca pratensis*, *F. rubra*, or *Dactylis glomerata*—the conditions are most inimical to the growth of *S. non-scripta* when additional nitrogen has been added to the sward early in the spring and the grasses have been allowed to grow unchecked. Conversely, maximum seasonal growth takes place when the shoots of the grasses but *not* those of the bluebell are frequently defoliated, and here adding nitrogen favours the growth of *S. non-scripta*. It is therefore concluded that when the grasses are not cut a high nitrogen level operates adversely by increasing the degree of shade cast by the grasses, while in the absence of shading due to differential defoliation competition for nitrogen comes into operation.

There is experimental evidence that large plants compete more successfully with the grasses than small plants which have not reached the flowering stage.

The intensity of competition is also dependent upon the dominant species in the sward. A comparison of the results for the two years (1938 and 1939) indicate marked seasonal differences. In 1938, a wet spring, small plants more than doubled their weight during the season, while in the following dry year comparable plants lost weight. In another experiment where 'flowering size' bulbs were planted in a *Festuca–Arrhenatherum* sward in 1937 and left for 4 years, the plants barely maintained their original weight over the period.

In an experimental assessment of the possible effects of grazing and trampling it has been found that the removal of the leaves and inflorescence well before the flowers open causes more injury than defoliation at a later stage. A double defoliation before and again during flowering brings about the maximum reduction (40–60 per cent.) in seasonal growth. When a *Festuca–Arrhenatherum* sward containing newly planted bluebells was defoliated once each spring, either at or before the flowering stage of the bluebell, then after a period of 4 years the final bulb weight was 31–43 per cent. less than the weight at planting. The adverse effects of trampling increase with the incidence and frequency. Trampling at intervals of a week significantly diminishes the growth rate more than trampling at 2- to 4-week intervals. Trampling at 10-day intervals from the time of leaf emergence significantly reduces the final weight more than when trampling is initiated 20 days later.

A comparison has been made of the effects of light intensity and mineral nutrient supply on the growth of plants from four different woodland habitats and from one site where the plants had been grown in the open. These samples from the five populations ('strains') showed striking differences in the seasonal growth rate. In full daylight the fastest growing strain gained 85 per cent. more in weight over the season than the slowest. Comparable differences were also found at lower lighter intensities, but the calculated light values at which no gain in weight should take place only varied between strains from 0·05 to 0·087 daylight.

For three strains which differed significantly in relative growth rate (efficiency index) it was found that while the linear regressions of net assimilation rate against the logarithm of light intensity did not differ significantly, yet similar regressions of leaf-area ratio (leaf area/total planting weight) showed marked differences. Apart, however, from these differences in leafiness, causing differences in the growth rate, the strains also varied in the time of shoot emergence in the spring and the commencement of active assimilation. There was no evidence that the strains varied in their response to different levels of nitrogen, phosphorus, and potassium.

With increasing bulb size, the subsequent relative gain in weight during the season decreases. It has been found that when plants are grown in full daylight this relationship can be empirically expressed as $Y = 1{\cdot}66x^{0{\cdot}543}$, where x is the initial bulb weight in the autumn and Y the increase in dry weight at the end of the following summer.

Two experiments in different years were carried out to determine whether plants which had been subject to varying levels of light intensity (0·2–

1·0 daylight) in one year grew in full daylight at different rates in the following year. In one experiment the light pre-treatments had no significant effects; in the other there was evidence that while the growth made during the whole season was unaffected, a greater proportion of the growth was made early in the season by the plants which had been most heavily shaded in the previous year.

On the basis of the present and past investigations, the conclusions reached are that the bluebell will be excluded from habitats where the following factors operate: (i) the mean light intensity between March and June is less than 0·05–0·07 daylight, or (ii) the ground is waterlogged or flooded during the winter, or (iii) the area is grazed or repeatedly trampled by large animals during the spring. The necessary conditions for the successful growth of the bluebell in grassland communities are: (i) the soils should remain moist in the spring but the drainage must be good; (ii) the mineral nutrient status of the soils should be low; (iii) the dominant grasses should combine a relatively prostrate habit with the characteristic of starting growth late in the spring; and (iv) the sward should not be grazed until after the flowering of *S. non-scripta*.

The bluebell will compete with *Pteridium aquilinum* outside woodland because (i) much of the seasonal growth is made in the high-light phase before the bracken fronds expand; (ii) bracken tends to exclude other competitive species; and (iii) dense bracken is associated with the absence of heavy grazing animals.

S. non-scripta is not susceptible to frosts and will grow in exposed situations (e.g. rock ledges) provided that the availability of water is adequate.

In woodland, shading *per se* can only be regarded as an inimical factor; the prevalence of the bluebell in deciduous woods is attributed to the absence of aggressively competitive species and more particularly to the protection provided by such habitats from grazing animals. Finally, it is advanced that precisely the same factors operate in confining other species to woodland.

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Metabolic Systems in the 'Root' of *Brassica napus* L.

BY

J. G. BOSWELL

(*The University, Sheffield*)

With three Figures in the Text

Abstract

This is a study of the effects of possible intermediary metabolites on the respiration of root tissue from *Brassica napus* using the Warburg micro-manometric technique. It is concluded that ascorbic acid is oxidized by two systems, one of which appears to be a direct oxidase and the other a dehydrogenase. No evidence of peroxidase activity was secured. A substantial fraction of the total respiratory activity was insensitive to cyanide and azide. The biologically important organic acids were oxidized with the production of carbon dioxide. Glutamic and aspartic acids were metabolized with great rapidity, glycine and alanine much more slowly. A scheme integrating these results is outlined and compared with the respiratory systems existing in potato.

Introduction

IT is stated in the literature that the root of *B. napus* L. does not contain the polyphenol oxidase system; this statement can be confirmed, for the addition of any water-soluble catechol derivative to the respiring tissue slices does not increase the rate of oxygen uptake.

Observations have therefore been made on the respiratory metabolism of this tissue. They are reported in three sections: the first is concerned with ascorbic acid oxidation; the second with catalase and peroxidase systems; while the third deals with the metabolism of certain biologically important compounds. In the final discussion an attempt is made to compare the metabolic processes in slices of the swede root and the potato tuber.

Technique

The roots were purchased locally without reference to variety or uniformity of supply. The tissue was cut into slices 0·025 in. thick and these were washed in running aerated tap-water for between 24 and 144 hours before use. Except when specifically stated to the contrary the slices were suspended in 4 ml. of a solution of potassium phosphate of pH 5·5–5·8 at 24° C. in Warburg micro-respirometer flasks. It was found that phthallate buffers had a depressor effect upon the rate of respiration and that other buffers of suitable pH value contained substances which served as metabolic substrates. For further details of technique reference should be made to Boswell and Whiting (1938) and Boswell (1945).

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With the following exceptions the chemical substances used were purchased from commercial sources: perbenzoic acid was prepared from benzoyl peroxide by the method of Gèza Braun (1933), hydrogen peroxide-urea by the method of Chia-Si Lu, Hughes, and Giguère (1941), oxaloacetic and dihydroxymaleic acids, Boswell (1945), and the α-ketoglutaric acid was a gift from Professor R. D. Haworth.

The rates of respiration were calculated as μl./g. dry weight/unit time. In certain experiments it was observed that the dry weight of the tissue at the end of the experiment was abnormally low. The cells appear to contain a considerable amount of water-soluble material which diffuses out into the surrounding medium, following an increase in cell permeability as the result of the presence of certain respiratory inhibitors or the complete destruction of the mechanism of permeability control by dilute H_2SO_4. The substances which inhibit respiratory activity can be divided into two groups. The first group contains malachite green and sodium monoiodoacetate which increase cell permeability. The second group contains potassium cyanide, sodium fluoride, and sodium azide which are without effect upon the permeability in concentrations which markedly reduce the rate of respiration. It would therefore appear reasonable to suggest that the control of cell permeability is located in a particular part of the respiratory mechanism and only when that particular system is inhibited does increased cell permeability develop with loss of cell contents. Malachite green and iodoacetate are dehydrogenase inhibitors and it would appear therefore that the permeability control mechanism is associated with dehydrogenase systems. It is of interest to note certain observations which have been recorded by other workers on the relationship between the accumulation and retention of dissolved substances within the cell and the action of dehydrogenases. Stiles and Jørgensen (1917) showed that potato slices lose electrolyte rapidly in the presence of certain substances including urethane, a known dehydrogenase inhibitor. From the report of Commoner, Fogel, and Muller (1943) it would appear that the four-carbon dicarboxylic-acid system, which is controlled by dehydrogenases, is linked with salt absorption. Lastly, there is the relationship between respiration and salt absorption demonstrated by Machlis (1944) using respiratory inhibitors such as iodoacetate.

In view of the effect of substances such as malachite green upon the dry weight of the tissue slices in those experiments in which they are used, a factor was calculated so that the respiratory activity could be determined in terms of the probable dry weight of the slices at the beginning of the experiment.

I. Ascorbic Acid Oxidation

Amounts of ascorbic acid up to approximately 2 mg. were added to about 1 g. fresh weight of washed slices in phosphate buffer solution and the effect of the addition on the rates of oxygen uptake and carbon dioxide output recorded. Fig. 1 illustrates the effect of adding 2·1 mg. and shows that even after 3 hours the stimulation of the respiration rate is maintained about 30

per cent. above the pre-addition value. The total excess oxygen uptake is 186 μl., equivalent to almost 1·4 atom oxygen per mol of added ascorbic acid. The terms 'excess oxygen uptake', 'excess carbon dioxide output', and 'excess R.Q.' are defined as the amount of each gas above the pre-addition level and the ratio between the two.

Even $4\frac{1}{2}$ hours after the addition of ascorbic acid the rate of oxygen uptake remains above the pre-addition level, and in fact no experiment has been continued to the point, if indeed such exists, at which the rate of oxygen uptake

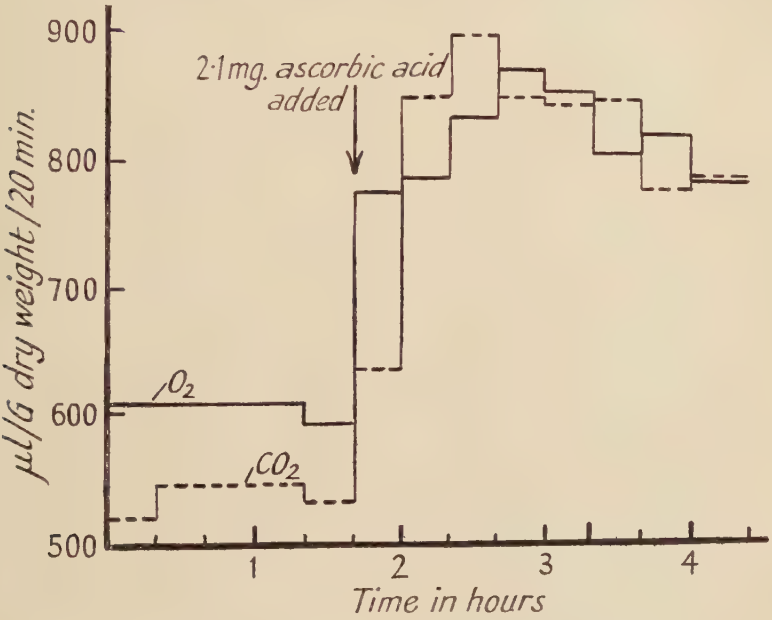


Fig. 1. Effect of ascorbic acid on respiration.

falls to the initial value. It is not possible therefore to calculate the excess oxygen uptake exactly in terms of the amount of ascorbic acid added, but it can be stated that for experiments of equal duration the larger the amount of ascorbic acid added the greater the excess oxygen uptake (Table I). There is some variation from day to day in the ascorbic acid oxidizing power of the tissue per unit dry weight which is in part related to the period during which the tissue has been washed prior to use, in part to the total respiratory activity of the tissue, and to other, as yet unrecognized, causes.

Not only is the rate of oxygen uptake increased by the addition of ascorbic acid but there is also an increase in the rate of carbon dioxide output; this increase is maintained at a value in excess of the pre-addition rate throughout the longest experiment, and the larger the amount of ascorbic acid added the larger is the excess carbon dioxide output. This increase is not, however, proportional to the increased oxygen uptake, and the excess R.Q. falls with increased additions of the acid.

TABLE I

Increased Respiratory Activity (in Excess of Pre-addition Value) following the Addition of Ascorbic Acid

Expt. No.	Amount of ascorbic acid added (mg.).	Duration of expt. (hrs.).	Excess O_2 uptake $\mu l.$	Excess CO_2 output $\mu l.$
	Slices washed for 2 days.			
49.93	0·74	3	30	25
	1·85	3	89	58
	2·00 + 2·00 mg. oxidized ascorbic acid	3	100	121
47.212	1·05	4	59	69
	2·10	4	107	77
	Slices washed for 5 days.			
46.612	0·84	4	82	54
	2·10	4	142	84

Experiments were conducted in which the ascorbic acid was partially oxidized before being added to the tissue. It is clear from Table I that in the presence of dehydroascorbic acid there is a substantial increase in the excess carbon dioxide output and only a small increase in the excess oxygen uptake.

These observations suggest that the oxidation of ascorbic acid by the tissue is linked to the stimulation of carbon dioxide production through a reaction between the dehydroascorbic acid produced and some substance within the tissue. Further, as a result of this reaction, it may be that ascorbic acid is produced and is available for re-oxidation.

Effects of malachite green and sodium azide

The mechanism of the oxidation of ascorbic acid and the relationship between ascorbic acid and the reactant within the tissue was further investigated using two respiratory inhibitors, malachite green and sodium azide.

Malachite green was added to give a final concentration of 1/4000 in the flask. This completely inhibited the respiration of the tissue after 3 hours. The values recorded in Table II show the effect on the respiration rate of the addition of malachite green and ascorbic acid separately and together.

Under the experimental conditions with ascorbic acid present in excess, the fact that malachite green reduced the rate of oxidation of the acid by one-third suggests that the ascorbic acid was being oxidized along two separate channels, one malachite-green sensitive and the other malachite-green insensitive, either by a direct oxidase or by a metallic, non-biological system. The complete inhibition of the oxidation by sodium azide, Table III, shows that the oxidation is wholly enzymic and that the oxidases concerned were azide sensitive.

TABLE II

Effect of Malachite Green and Ascorbic Acid on Respiration (in Excess of Pre-addition Value)

	O_2				CO_2			
		μl. excess.				μl. excess.		
Time in hours.	% inhibition of O_2 uptake. Malachite green alone.	Ascorbic acid alone.	Malachite green and ascorbic acid.	% inhibition.	% inhibition of CO_2 output. Malachite green alone.	Ascorbic acid alone.	Malachite green and ascorbic acid.	% inhibition.
1st	49	68	45	34	11	64	7	89
2nd	75	50	31	38	83	62	18	71
Total	—	118	76	36	—	126	25	80

TABLE III

Effect of Sodium Azide and Ascorbic Acid on Respiration (in Excess of Pre-addition Value)

	O_2				CO_2			
		μl. excess.				μl. excess.		
Time in hours.	% inhibition of O_2 uptake. Na azide alone.	Ascorbic acid alone.	Na azide and ascorbic acid.	% inhibition.	% inhibition of CO_2 output. Na azide alone.	Ascorbic acid alone.	Na azide and ascorbic acid.	% inhibition.
1st	64	40	38	5	33	23	24	+4 (Not significant)
2nd	79	34	10	70·6	62	27	2	92·6
3rd	87	25	0	100	74	31	0	100
Total	—	99	48	52	—	81	26	68

The malachite green added to the tissue slices had only a slight inhibitory effect on their carbon dioxide output during the first hour; in fact, in certain experiments the initial effect is slightly stimulating, the inhibition only appearing at a later stage. During the first hour the effect of the malachite green is probably complex, a combination of the inhibition of the dehydrogenase systems—Quastel and Wheatley (1931)—masked by a continued and uninhibited decarboxylation of the reserve of α-keto acids with the additional changes which result from the disturbance of the permeability relations of the cells. The data in Table II show, however, that the excess carbon dioxide which follows the addition of the ascorbic acid to the tissue is greatly reduced in the presence of malachite green. This suggests that the evolution of the carbon dioxide is either directly linked with or follows upon a dehydrogenase reaction. It may be that the excess carbon dioxide is the product of that part of the oxidation system which is malachite-green sensitive; alternatively, the excess carbon dioxide may result from a reaction between the dehydroascorbic acid and some H-donator in the tissue. This latter possibility would agree with a suggestion made earlier as the result of the first group of experiments. The problem of the sources of the excess carbon dioxide will be considered later in the light of other results.

Sodium azide was added to the slices of tissue to give a final concentration of M/400 and in Table III are set out the data showing the azide action calculated in the same way as those for malachite green recorded in Table II. The form of the oxygen uptake-time relation for the tissue slices in the presence of sodium azide suggests that the oxygen uptake is the result of the action of two oxidases or groups of oxidases. During the first 20 minutes following the addition of the azide the oxygen uptake amounts to only 50 per cent. of the pre-addition value, while during the second 20 minutes the residual oxygen uptake has fallen to 30 per cent. Thereafter the rate drifts slowly downwards, but in no experiment was the whole of the oxygen uptake completely inhibited, a basic rate of about 10 per cent. remained even after several hours. The second phase of the azide inhibition may show the existence of a relatively insensitive oxidase or this oxidase be wholly resistant to azide, the manifestation of its activity decreasing owing to the disorganization of associated systems following the complete inhibition of the azide-sensitive oxidase. The azide inhibition of the carbon dioxide output is such that the R.Q. approximates to 2 throughout the experiment.

During the first hour following the addition of the ascorbic acid to the tissue the excess oxygen uptake and excess carbon dioxide output are only slightly modified by the presence of sodium azide. From the second hour onwards the inhibition develops rapidly and is finally complete. The very small degree of inhibition of ascorbic acid oxidation during the first hour may be an example of the protective action of the excess of substrate on the activity of the enzyme in the presence of an inhibitor, the protection being substantial until a considerable concentration of azide has developed within the cells and there has been a considerable reduction in the amount of ascorbic acid present.

During rapid development of inhibition the ascorbic acid-stimulated excess oxygen uptake and excess carbon dioxide output are affected to the same degree. Thus it would appear probable that carbon dioxide production is linked directly with the azide-sensitive oxidase systems rather than with that part of the oxidation of ascorbic acid which occurs through the agency of a dehydrogenase. The latter would be only indirectly affected by azide and so might continue to operate for some time after the direct oxidase system was partially inhibited. This points to the production of carbon dioxide being due to a reaction between an H-donator in the tissue and the dehydro-ascorbic acid formed by the action of a direct oxidase.

While evidence has thus accumulated of the capacity of the tissue to oxidize ascorbic acid, of the systems by which this is achieved, and of the mechanisms involving carbon dioxide output, it has not been possible to determine what, if any, part ascorbic acid and its oxidase play in tissue respiration.

Sodium diethyl-dithio-carbamate is a highly specific reagent for traces of copper, and since ascorbic acid oxidase is a copper-protein complex its activity might well be inhibited by the carbamate. However, the latter decomposes rapidly in the presence of a phosphate buffer at pH 5·5 with the evolution of some gas. Hence, while some inhibition of oxygen uptake by the tissue appears to take place in the presence of carbamate, the gaseous changes which result from carbamate decomposition make it impossible to secure results from which conclusions of any value can be drawn.

Possible hydrogen donators

If ascorbic acid and its oxidase form part of a cyclic system whereby oxygen is absorbed and carbon dioxide given out, it would be of interest to examine the nature of the H-donator by which the dehydro-ascorbic acid is reduced and the cyclic action of the system maintained. In view of the results obtained by James and his co-workers (1944) on the ascorbic acid-oxidase system in barley leaves, certain experiments were carried out using lactic and malic acids and hexose diphosphate. The results are set out in Table IV.

TABLE IV

Effect of certain H-donators on Ascorbic Acid Oxidation (*Values in Excess of Pre-addition Value*)

Expt. No.	Ascorbic acid alone.		Additional H-donator.		Ascorbic acid after additional H-donator.	
	O_2	CO_2	O_2	CO_2	O_2	CO_2
			μl. excess.			
47.412						
Lactic acid . .	185	214	24	43	113	56
Malic acid . .	—	—	38	79	133	95
48.411						
Hexose diphosphate .	162	64	0	0	155	19

In this series of experiments the H-donator to be tested was added to the tissue followed 1 hour later by 2·0 mg. of ascorbic acid. In all cases the excess

oxygen uptake following the addition of ascorbic acid was less in the presence of the possible H-donators than in their absence. With malic and lactic acids which stimulate the respiratory activity of the tissue the total excess oxygen uptake for both H-donator and ascorbic acid was less than that for the ascorbic acid alone. The excess carbon dioxide output values were reduced to an even greater extent than those of the excess oxygen uptake, which suggests that the added substances actually reduced the supply of naturally occurring H-donator, thereby slowing down the rate at which the cyclic oxidation and reduction of the ascorbic acid occurred.

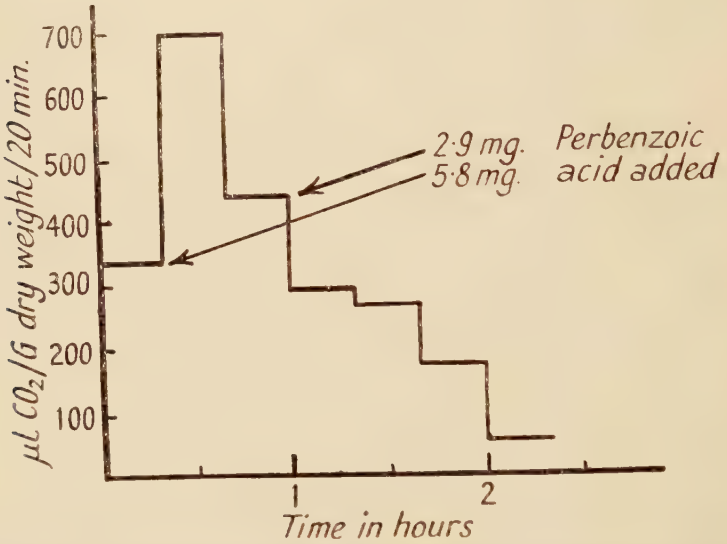


FIG. 2. Effect of perbenzoic acid on carbon dioxide output under anaerobic conditions.

It is not proposed to consider these observations further in this paper since it is clear that the gaseous exchanges are the aggregate of a complex group of reactions. An extensive biochemical investigation has been started to secure data from which further analysis of the mechanisms may be possible.

II. Catalase and Peroxidase Systems

As expected from all previously reported observations, when small amounts of hydrogen peroxide were added to tissue slices under anaerobic conditions the peroxide was decomposed by catalase. This also occurred when the hydrogen peroxide-urea complex was used. Clearly while this complex forms stable crystals it is strongly dissociated in aqueous solution. The possibility was considered of using compounds which might be decomposed slowly in the presence of the tissue with the liberation of what might be considered to be nascent hydrogen peroxide. Inorganic peroxides and percarbonates are unstable within the pH limits imposed by the presence of living tissues. Further, ammonium persulphate had no significant stimulating effect upon

the rate of carbon dioxide output when added to the tissue under anaerobic conditions. It was therefore necessary to consider the use of organic compounds. Perbenzoic acid was prepared and added to the tissue under anaerobic conditions. Fig. 2 shows that there was an initial stimulation of the carbon dioxide output, that this was followed quickly by a steep fall, and that this fall was not arrested by a second addition of perbenzoic acid. The low level of the carbon dioxide output at the end of 2 hours suggested that the perbenzoic acid had had a lethal effect upon the tissue. In the absence of this substance slices of swede root endure anaerobic conditions for several hours without any marked fall in the carbon dioxide output. Moreover, the bleached appearance, the flaccid state of the slices, and their low dry weight/volume ratios all support the view that the perbenzoic acid is toxic to the tissue.

TABLE V

The Effect of Sodium Dihydroxymaleate alone and in combination with certain Respiratory Inhibitors

Amount added (mg.).	Inhibitor.	Excess gas (μl.).	
		O_2	CO_2
2·05	None	100	160+88 bound
1·00	,,	52	67+13 ,,
1·23	,,	48	75 total
1·23	Malachite green 1/4000	21	63 ,,
1·55	None	65	120 ,,
1·55	Sodium azide M/400	60	124 ,,

In view of the statement that peroxidase and dihydroxymaleic acid-oxidase are identical systems it is proposed at this point to report certain observations on the effects of this acid on swede root. The addition of disodium dihydroxymaleate stimulates both the oxygen uptake and carbon dioxide output (Fig. 3). The stimulation of gaseous exchange is only of short duration and after about 2 hours the rates have returned to the pre-addition levels. This does not suggest that the acid takes part in any cyclic redox system. The values recorded in Table V show that the excess oxygen uptake does not in any case reach the theoretical value of $\frac{1}{2}$ mol oxygen for each mol of acid added, still less the larger values which might be expected with a substance taking part in a cyclic system. Determinations during the course of the metabolism of the acid show that there is a substantial increase in the amount of carbon dioxide bound in a form which can be decomposed with dilute acid; from which it may be deduced that during the experiment there is an increase in the basicity of the contents of the flask; this would occur if the salt were decomposed by decarboxyation with the liberation of sodium ions. The sodium salt of dihydroxymaleic acid appears to be oxidatively decarboxylated by a system which is partially inhibited by malachite green but which is wholly insensitive to sodium azide. There is no evidence that the dihydroxymaleate forms part

of a cyclic redox system, and since the oxidation is azide-insensitive it cannot involve peroxidase or any other enzyme with a heavy metal prosthetic group.

Effects of cyanide

Following upon consideration of these oxidase systems it is of interest to note the effect of cyanide upon the respiration rate of slices of swede root (Table VI). Some observations made using potato tuber and carrot root

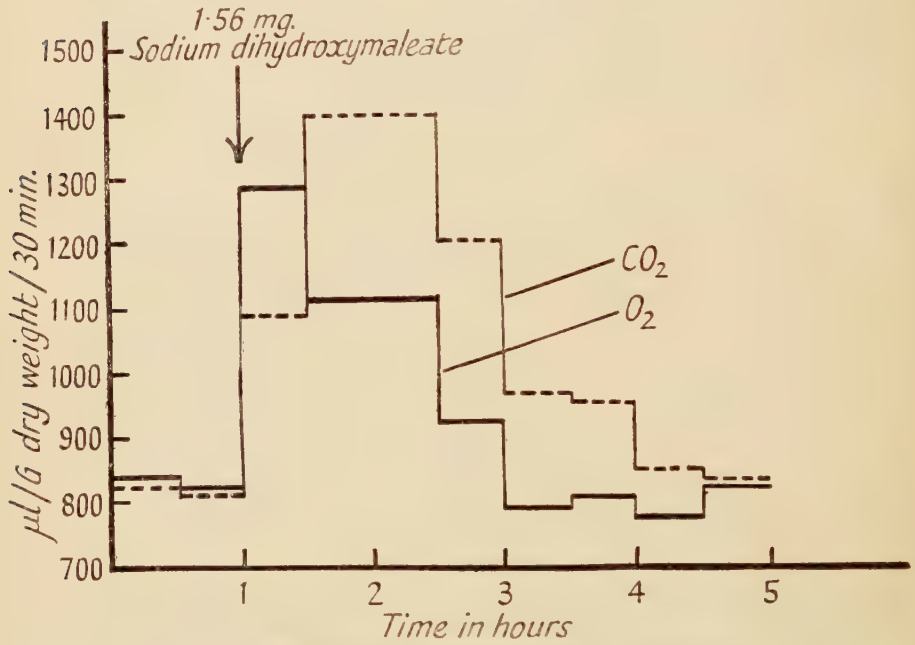


FIG. 3. Effect of sodium dihydroxymaleate on respiration.

slices are included for purposes of comparison (Table VII). In those flasks which contained sodium hydroxide in the centre cup the calculated amount of cyanide was mixed with the soda solution.

A double effect of cyanide upon respiration of swede slices can be noted, namely, that low concentrations stimulate oxygen uptake and carbon dioxide output while higher concentrations have an inhibitory effect upon both. The increase in the percentage inhibition of carbon dioxide output when M/240 KCN is used in comparison with the inhibition at M/48 is as yet unexplained, but is a regular and well-defined feature of these experiments.

The observations in Table VII were made using maincrop potatoes; with early varieties the general effects are the same but concentrations which just depress oxygen uptake and carbon dioxide output in the main crop while depressing the oxygen uptake actually stimulate the carbon dioxide output of the early varieties. While the results set forth in Tables VI and VII cannot be closely compared, as they are not for identical time intervals or concentra-

TABLE VI

Effect of KCN *on the Respiration of Swede Root Slices*

Final concentration of KCN.	Duration of experiment (hrs.).	Respiration rate during final hour as percentage of 'tissue only' value.	
		O_2	CO_2
M/48	4	33·0	55·0
M/240	4	42·7	49·2
M/800	4·5	119·5	126·0
M/2000	4·5	113·5	109·5

TABLE VII

Effect of KCN *on the Respiration Rate of Slices of Potato Tuber and Carrot Root*

Tissue.	Final concentration of KCN.	Respiration rate during 2nd hour after addition as percentage of the 'tissue only' value.	
		O_2	CO_2
Potato . .	M/500	45·5	85·8
	M/1000	78·8	124·0
	M/5000	93·8	129·0
Carrot . .	M/400	32·2	89·2
	M/1000	35·0	130·0

tions, nevertheless certain general conclusions can be drawn. With concentrations greater than M/500 KCN there is always a strong inhibition of oxygen uptake, indicating that part at least of the direct oxidase systems concerned contain a heavy metal prosthetic group. This is in agreement with the existence in the potato and carrot of the polyphenol-oxidase system and with the observation that in the slices of the former at least two-thirds of the respiratory activity is controlled by that system. While the oxygen uptake of the swede tissue is partially inhibited by such concentrations of cyanide, there are nevertheless very clearly marked differences between the reaction of this tissue and those of carrot and potato. First, the rate of oxygen uptake by the swede is much less sensitive to cyanide than that of the other tissues, and second, the rate of oxygen uptake by the swede is actually stimulated by concentrations of cyanide of less than M/800 while with potato M/5000 has a distinctly inhibiting effect. This suggests that the swede contains a cyanide-insensitive direct oxidase system which may or may not be directly stimulated by cyanide but which plays a major part in the oxygen uptake mechanism of the normal slices. It cannot be assumed that such a system is absent from the potato and carrot, but if present it is masked by the dominance of the cyanide-sensitive system.

The effect of cyanide upon the carbon dioxide output from these three tissues is much more uniform; concentrations greater than M/500 are inhibitory, but lower concentrations stimulate the output strongly. In the case of

the potato and carrot cyanide possibly acts upon the rate of production of the intermediates which on decomposition give carbon dioxide. It is improbable that the cyanide effect on the potato slices can be due to its action upon the starch-sugar equilibrium, since there is no evidence to support the view that the limiting factor in the respiration of sliced tissue is the sugar content. Hanes and Barker (1931) using whole potatoes found that the stimulation of the rate of respiration by very dilute HCN in the air-stream was correlated with the increased sugar content of the tubers. It was suggested from their observations upon the effect of cyanide in stimulating the activity of amylase *in vitro* that this was the key to the stimulating effect of cyanide upon the respiration rate of the tubers. Other observations have already made it clear that while the actual respiration mechanisms in the whole tubers and in the slices are probably alike, the factors limiting the rates in the two tissues are quite different. For example, the dominant position of protein metabolism in the respiratory activity of slices of potato tuber has already been demonstrated (Boswell, 1945). With swedes there exists a close relationship between the stimulating effect of cyanide upon the rate of oxygen uptake and of carbon dioxide output and it is not possible to determine the identity of the limiting reaction which is stimulated by the addition of cyanide. It is clear also that in the swede at cyanide concentrations of about M/800 any inhibitory effects are completely masked by the strong stimulation of both oxygen uptake and carbon dioxide output.

III. Other Metabolites

In other experiments a wide range of substances was used in order to obtain information concerning their activity as possible intermediate metabolites. Because of the technique of suspending the tissue slices in a potassium phosphate solution and of allowing a time interval of at least 1 hour before the first readings of the manometers were taken, the salt effect which has been studied with carrot slices, if it occurs with swede tissue, is not recorded. Whiting (1938) found no salt effect with potato tissue. The results which are set forth in the following tables show therefore only the effect of the organic ions of the compounds used.

Organic acids of the tricarboxylic acid cycle

The first group contained the four-carbon dicarboxylic acids and certain substances associated with them in what may be described as the 'citric acid cycle' without prejudging the issue as to whether or not the cycle actually exists in the tissue. The acids were added to tissue suspended in buffers at two separate pH values; the results are recorded in Table VIII.

It is clear that the tissue is capable of utilizing these acids in some part of its metabolic system and that with the exception of citric acid the substances are metabolized more actively at pH 4·5 than at 5·5. This may be due either to a connexion between pH and permeability or to the action of pH in determining the degree of ionization of the organic acids added to the external

solution, with the consequent regulation of the concentration of the effective ion. Of course, a combination of both effects may occur.

TABLE VIII

Effect of Certain Organic Acids upon the Rate of Respiration of Slices of Swede Root (Values in Excess of Pre-addition Rate)

Acid added	Citric.	α-keto-glutaric.	Succinic.	Malic.	Oxaloacetic.	Pyruvic.
			Buffer pH 5·5			
Concentration in flask as $M \times 10^{-3}$/l.	4·85	3·04	4·24	4·48	3·05	5·25
Excess gas in μl. during 2 hrs.						
O_2	58	4	13	34	11	9
CO_2	169	29	24	76	49	27
Excess R.Q.	2·9	7·2	1·8	2·2	4·5	3·0
			Buffer pH 4·5			
Concentration in flask as $M \times 10^{-3}$/l.	2·29	3·04	3·20	2·98	3·65	6·03
Excess μl. during 2 hrs.						
O_2	102	121	61	106	24	32
CO_2	172	218	107	186	153	127
Excess R.Q.	1·68	1·80	1·75	1·75	6·4	4·0

The high excess R.Q. values for pyruvic and oxaloacetic acids at both pH levels indicates the activity of enzymes which decarboxylate these acids if indeed the decarboxylation of oxaloacetic acid is an enzymic reaction. The decomposition of α-ketoglutaric acid is recorded in the literature as an oxidative decarboxylation with an R.Q. of 2. From Table VIII it may be seen that a very wide gap exists between the excess R.Q. at pH 5·5 and that at 4·5 when this acid is used as a metabolite. This may be explained if consideration is given to the fact that at the higher pH of 5·5 the rate of metabolism is low, the gaseous exchanges being very small in comparison with the values at pH 4·5. If in the dehydrogenation of the acid the hydrogen is retained even in part by the initial hydrogen acceptor and not passed immediately and completely to a terminal direct oxidase system, then the recorded oxygen uptake will not measure the oxidation of the acid and the excess R.Q. will be too high. Now at pH 5·5 the excess gaseous exchange values are so small that the retention of only a little hydrogen by an intermediate acceptor would seriously distort the excess R.Q. value, while at 4·5 a similar retention would have very little effect upon the excess R.Q. value. For example, an increase in the recorded oxygen uptake of 12 μl. following the addition of α-ketoglutaric acid at pH 5·5 would reduce the excess R.Q. from 7·2 to 1·8, while at pH 4·5 the excess R.Q. would be reduced only from 1·8 to 1·64. This point is of general application, namely, that the excess oxygen uptake is not necessarily a complete measure of the oxidation of the added substrate and also that where the total gaseous exchanges are small the magnitude of the excess R.Q. value has no very great significance. No importance should therefore be attached to the differences between the

excess R.Q. values recorded for oxaloacetic and pyruvic acids at pH 4·5 and 5·5 since in both cases the excess oxygen uptake values at pH 5·5 are too small to have been an accurate measure of the oxidation of these substrates. The only result which cannot be explained on this basis is with citric acid, where the values at both pH are large enough to be significant and yet a very considerable difference exists between the excess R.Q. values.

With citric, α-ketoglutaric, succinic, and malic acids at pH 4·5 the excess gaseous exchanges are sufficiently large to make the values significant and the uniformity of the excess R.Q. values is most striking. When consideration is given to the excess R.Q. values which result from the complete oxidation of these acids it is clear that the reactions into which they enter involve predominantly decarboxylation with the formation of organic residues which are not completely oxidized. It cannot be ruled out that these acids act as catalysts, but there is no evidence of the existence of the citric acid cycle. This can only be further examined when data are available of the chemical reactions into which these substances enter. The results obtained using fumaric acid are almost identical with those for malic acid and leave no doubt as to the existence of an active fumarase.

The prior addition to the tissue of malachite green to give a final concentration of 1/1500 completely inhibited the stimulation of respiratory activity which follows the addition of citric, α-ketoglutaric, succinic, fumaric, and malic acids to the tissue in the absence of the dyestuff. This shows that the excess oxygen uptake which follows the addition of these acids to normal slices is associated with dehydrogenases. It appears that the decarboxylation which yields the carbon dioxide is either preceded by or coincident with dehydrogenation. With oxaloacetic and pyruvic acids the prior addition of the inhibitor prevents any stimulation of the oxygen uptake on the subsequent addition of the acids, but the excess carbon dioxide output is only very little less than the value secured in the absence of the inhibitor. This suggests that whatever part these acids may have in a metabolic system, decarboxylation can occur independently of dehydrogenation and also that the excess oxygen uptake which follows their addition to the normal tissue is associated with a dehydrogenase system. This does not eliminate the possibility that in normal tissue the metabolism of pyruvic acid is an oxidative decarboxylation.

Amino-acids

The second group of substances examined consists of the amino-acids, glutamic and aspartic acids, alanine, glycine, and tyrosine, and the amide asparagine. Observations made using these substances are recorded in Table IX. It may be noted that Boswell (1945) has recorded that this group of substances had no effect upon the rate of respiration of washed slices of potato tuber.

These values were obtained using a buffer at pH 4·5; at 5·5 the excess oxygen uptake and carbon dioxide output values with glutamic and aspartic acids and asparagine were all very much lower and the excess R.Q. values larger, while

TABLE IX

The Effect of Certain N *Compounds upon the Rate of Respiration of Slices of Swede Root (Values in Excess of Pre-addition Rate)*

Acid added	Glutamic	Aspartic	Alanine	Glycine	Tyrosine	Asparagine
Concentration in flask as $M \times 10^{-3}$/l.	4·15	4·17	5·75	6·8	1·50	4·29
Excess μl. during 2 hrs.						
O_2	106	80	12	10	−14	41
CO_2	180	142	13	7	−17	71
Excess R.Q.	1·71	1·78	1·08	0·70	—	1·74

those with alanine and glycine were little affected. It is to be noted that while glutamic and aspartic acids and asparagine stimulate the rate of respiration to a very considerable extent, alanine and glycine have very little effect and tyrosine is slightly inhibitory, the rate of respiration following addition falling below the 'tissue only' level. The difference between aspartic acid and asparagine as stimulators of respiratory activity is probably due to the permeability relations of the two substances. To examine the possibility that the metabolism of glutamic and aspartic acids is through an amino-acid oxidase, these amino-acids were added to tissue whose respiratory activity had been partially inhibited by the prior addition of malachite green. The results are set out in Table X. It is clear that the oxidations of glutamic and aspartic acids are achieved by a dehydrogenase system and not by a direct oxidase. It is of interest that glutamic acid is capable of partially removing the inhibition of carbon dioxide output resulting from the action of malachite green, an action comparable to that of pyruvic acid. The non-oxidative decarboxylation of pyruvic acid is a well-known reaction and it would appear therefore that under these experimental conditions glutamic acid is being decarboxylated in a non-oxidative reaction. This is in accord with the results of Schales and Schales (1946), who have prepared a glutamic acid decarboxylase from a wide range of plants including turnip and swede.

TABLE X

The Action of Certain Substances upon the Degree of Inhibition of Respiratory Activity caused by Malachite Green in a Final Concentration of 1/15,000

	No organic compound.		Glutamic acid.		Aspartic acid.		Pyruvic acid.	
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
Tissue only	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
Malachite green added	0·54	0·85	0·54	0·85	0·55	0·86	0·55	0·92
Organic compound added	0·28	0·42	0·32	0·53	0·33	0·53	0·33	0·51
	0·17	0·28	0·13	0·64	0·13	0·24	0·09	0·89
	0·13	0·17	0·13	0·30	0·11	0·10	0·10	0·70

The respiration rates are shown as ratios of the initial 'tissue only' values and pyruvic acid is included for the purpose of comparison.

Other organic acids and acetaldehyde

The third group of substances includes lactic, tartaric, oxalic, and formic acids and acetaldehyde. Of the four acids formic has an inhibitory action on both oxygen uptake and carbon dioxide output, the treated tissue becomes flaccid, and the dry weight per volume ratio is low. Table XI shows the stimulating effect on respiration of lactic, tartaric, and oxalic acids with particular reference to the influence of the pH at which they are applied. The

TABLE XI

The Effect of Certain Organic Acids on the Rate of Respiration of Slices of Swede Root (Values in Excess of Pre-addition Rate)

pH.	Acid added.	Amount (mg.).	Excess in μl. O_2 uptake.	Excess in μl. CO_2 output.	Excess R.Q.	Duration of expt. (hrs.).
4·5	Oxalic	2·1	33	120	3·6	2
6·0		2·0	8	48	6·0	4
4·5	Tartaric	2·5	70	156	2·2	4
6·0		2·0	10	30	3·0	4
4·5	Lactic	2·0	39	90	2·3	3
6·0		2·1	13	30	2·3	3

nature of the metabolic processes in which these acids are involved remains to be investigated later, but certain points may be noted. The high excess R.Q. with oxalic acid suggests complete oxidation to carbon dioxide and water; little significance can be attached to the value at pH 6, since with an excess oxygen uptake of only 8 μl. the accurate determination of oxidation is impossible. The use of malachite green in the usual way shows that in all cases the oxidation is through a dehydrogenase system and the uptake of oxygen and the output of carbon dioxide are the products of closely linked reactions.

Acetaldehyde depresses irreversibly the rate of oxygen uptake immediately after addition. The rate of carbon dioxide output is strongly stimulated in the early stages, the R.Q. rising to about 2. Later, carbon dioxide output is reduced, and after 3 hours both oxygen uptake and carbon dioxide output are below the 'tissue only' value and the R.Q. has fallen to 1·25. When acetaldehyde is added to the tissue after malachite green the inhibition of the oxygen uptake is much more rapid than with the malachite green alone, there is not a proportionate reduction in the output of carbon dioxide and the R.Q. rises steadily. It is of interest to note that as in the case of the potato tuber tissue acetic acid has a depressor action on the rate of respiration and a similar effect with barley roots has been observed.

Effect of iodoacetic acid

Iodoacetic acid has been shown to inhibit several dehydrogenases by acting on the —SH group. When added to tissue slices in a final concentration of $1{\cdot}25 \times 10^{-2}$M, inhibition of respiratory activity is complete after $1\frac{1}{2}$ hours, and at $1{\cdot}25 \times 10^{-3}$M the oxygen uptake is reduced to 10 per cent. and the carbon

dioxide output to 25 per cent. of the pre-addition values after 3 hours, all at pH 5·5. The degree of inhibition is related to the pH value, decreasing as the acidity decreases. Citric, succinic, pyruvic, aspartic, and glutamic acids were added to tissue treated half an hour earlier with sodium monoiodoacetate in a final concentration of $1{\cdot}25 \times 10^{-3}$M. With citric, succinic, and aspartic acids the inhibition of oxygen uptake and carbon dioxide output was not removed. With pyruvic and glutamic acids only the carbon dioxide output was stimulated to a considerable extent and that only temporarily, in the case of glutamic acid the carbon dioxide output being above the pre-inhibition value for a short time. These observations are in line with those made previously with another dehydrogenase inhibitor, malachite green, from which it was deduced that with citric, succinic, and aspartic acids the production of carbon dioxide is linked with dehydrogenases, while with pyruvic and glutamic acids direct decarboxylation may occur.

Effect of sodium fluoride

Sodium fluoride in a final concentration of $1{\cdot}25 \times 10^{-2}$M was used as a respiratory inhibitor. After $4\frac{1}{2}$ hours the rate of oxygen uptake was 29 per cent. of the pre-addition level and the R.Q. had risen to 1·58. The data set forth in Table XII show that the acids of the 'citric acid' cycle are all capable of reducing the inhibition of respiration produced by the addition of sodium fluoride. Except with succinic and citric acids the rate of respiration is raised above the pre-addition level, but with these two acids only the rate of carbon dioxide

Table XII

Respiration Inhibited by the Addition of Sodium Fluoride to a Final Concentration of $1{\cdot}25 \times 10^{-2}$*M Forty Minutes before the Addition of the Organic Acids*

Acid	Malic.	Pyruvic.	Succinic.	Citric.	Oxaloacetic.
Concentration $10^{-3} \times$ M	4·6	5·8	4·6	4·6	4·5
Excess gaseous exchange in μl. during 2 hours over the respiration rate following the addition of fluoride.					
O_2	112	120	109	81	91
CO_2	103	124	94	96	153
R.Q.	0·92	1·04	0·86	1·18	1·68
Excess gaseous exchange in μl. during 2 hours over the respiration rate before the addition of fluoride.					
O_2	57	66	—	—	10
CO_2	75	97	58	22	65
R.Q.	1·32	1·47	—	—	6·5

output passes this level. That succinic acid should be one of the two less effective acids is of interest in view of Borei's (1945) observation that while sodium fluoride inhibits the enolase in the hexosephosphorylation cycle it also inhibits the cytochrome-oxidase system. Since these acids remove or reduce the fluoride inhibition it would appear that their part in the respiration system follows upon the phosphorylation cycle, the products of glycolysis being the

substrates for the organic acid metabolic reactions. This would be additional support for the schemes suggested by Gregory and Sen (1937) and Steward and Street (1947).

Comparison between the data in Tables VIII and XII reveals the different metabolic reactions in which the organic acids are involved in the presence and absence of sodium fluoride. In the presence of fluoride, while the rates of metabolism are of the same order as in its absence at pH 4·5, the excess R.Q. values are much smaller even in the case of oxaloacetic acid. This supports the view, based upon the high R.Q. values for fluoride-treated tissue, that fluoride inhibits the production of oxidizable substrates. These can be replaced by certain organic acids and the fact suggests that a considerable proportion of the respiratory activity of the normal swede slices is directed through the metabolism of organic acids.

Discussion

It is proposed in this section to consider the observations which have been made using the swede 'root' (*Brassica napus*) and to compare them with those recorded by Boswell and Whiting (1938) and Boswell (1945) from the potato tuber.

It must be emphasized that any conclusions set forth are only valid with reference to washed, sliced tissue, and that they do not necessarily give a picture of the respiratory mechanism for the whole tuber or root. From the work of Steward it is clear that slicing and washing with adequate aeration alters the balance of the respiratory mechanisms. This is partly due to the increased oxygen supply and partly to the initiation of certain chains of reactions associated with meristematic activity. For example, those which maintain the constant level of protein nitrogen in the whole mature organ become distorted in such a way that the rate of protein synthesis is stimulated without a proportionate increase in the rate of protein catabolism; thus an increased protein content results. Further, it is clear from the work of Berry and Steward (1934) that different storage tissues react to wounding with widely different intensities.

When attempting to draw a comparison between the respiratory mechanism of slices of swede root and of potato tuber certain metabolic differences are clear. The most important of these relates to the nature of the direct-oxidase systems through which hydrogen is transferred to molecular oxygen. In accordance with previous reports it is confirmed that swede root does not contain the polyphenol oxidase system. A complete identification of the direct oxidase systems in swede root has not yet been achieved. The form of the respiration-time curve following the addition of ascorbic acid, excess oxygen uptake values greater than one atom oxygen per mol of ascorbic acid added, the effects of dehydroascorbic acid, of sodium azide, and malachite green, all suggest that ascorbic acid takes part in a cyclic redox system. In this the uptake of oxygen is associated with the activity of a direct oxidase and the liberation of carbon dioxide is connected with the reduction of dehydro-

ascorbic acid. No evidence is available as to the nature of the H-donator involved in this reduction. Under the experimental conditions with an excess of ascorbic acid present there is evidence that part of the added ascorbic acid is oxidized by a dehydrogenase system. This is comparable to that which occurs in the potato tuber, where ascorbic acid appears to be the co-enzyme of a redox system which does not directly involve oxygen.

The nature of the other direct oxidase systems in the tissue can as yet only be inferred from their reactions to known specific inhibitors. It is clear that some part of these systems involves one or more enzymes which do not contain a heavy metal in their prosthetic group since they are insensitive to both azide and cyanide. All attempts to determine the position of peroxidase in the respiratory system have failed; it is also certain that as in the potato tuber dihydroxymaleic acid does not form part of a terminal oxidase system.

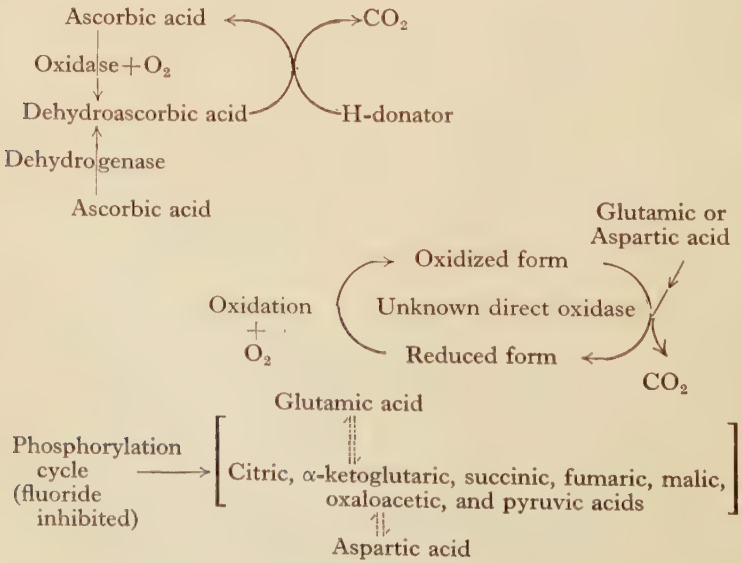
The effect of cyanide in small concentrations in stimulating the rates of both oxygen uptake and carbon dioxide output of swede slices raises the question of the mechanism of the stimulation. In concentrations which do not inhibit enzyme activity, it cannot be ruled out that the cyanide serves as an oxidizable substrate with resulting stimulation of the rate of gaseous exchange. With the potato tuber and carrot root the oxygen uptake mechanism is much more sensitive to cyanide; this is to be expected from the dominant position of the polyphenol oxidase among the terminal oxidases. It may well be that the absence of any stimulation of oxygen uptake in these two tissues is a resultant effect, the marked inhibition of one oxidase system being not wholly compensated for by the stimulation resulting from cyanide operating as a metabolite, which is a possibility in view of the stimulation of the carbon dioxide output.

As with slices of potato tuber the results showed that slices of swede root metabolized the four-carbon dicarboxylic-acids together with pyruvic, citric, and α-ketoglutaric acids. These reactions were apparently controlled by dehydrogenases. The data obtained by adding the organic acids to fluoride-treated tissue indicates that these acids form a metabolic system which uses as an initial substrate the products of the phosphorylation cycle. The respiratory activity of potato tuber slices is only inhibited by concentrations of fluoride which render the tissue somewhat flaccid; this insensitivity is not unexpected since it has been shown that the polyphenol oxidase system is insensitive to fluoride. The evidence available does not permit a decision to be reached as to the existence in the tissue of a system comparable to the tricarboxylic-acid cycle.

In contrast to slices of the potato tuber, the respiratory activity of slices of the swede root is stimulated by the addition of amino-acids and in particular by glutamic and aspartic acids. The metabolism of these two acids, which is very much more vigorous than that of glycine and alanine, may occur along two lines. Glutamic and aspartic acids may take part in transamination systems, and the gaseous exchanges which follow their addition to the tissue may be due to the metabolism of the resulting α-keto acids. The excess R.Q. values for the metabolism of glutamic acid and of the α-ketoglutaric acid which

would result from the transamination are so close together that this line of metabolism may occur. With aspartic acid the excess R.Q. values for acid and the corresponding α-keto acid, oxaloacetic, are so far apart that it is unlikely that aspartic acid is metabolized through transamination.

Since it has been shown that these amino-acids are oxidized by the action of dehydrogenases, the alternative line for both amino-acids would be analogous to the scheme set forth previously for the potato tuber. These acids would serve as H-donators to some direct oxidase system, carbon dioxide being evolved in the process and the increased rate of oxygen uptake being due to the increased rate of production of the reduced phase of the oxidase system. The difference between the reactions of potato and swede slices to the addition of these amino-acids would reflect the different factors which limit the rate of respiration in the two tissues. In the potato tuber slices it has been shown that the factor limiting the rate of respiration is the amount of the naturally occurring polyphenol. When this is supplemented by the addition of caffeic acid the supply of amino-acid H-donator becomes the limiting factor and only in that state does the addition of glutamic and aspartic acids stimulate the rate of respiration. If in the original slices the factor limiting the rate of respiration had been the supply of the amino-acid which functions as the H-donator and not of the natural polyphenol, then the addition of the amino-acid would have stimulated the respiratory activity. Glutamic and aspartic acids added to swede tissue have exactly this effect and this is compatible with them acting as H-donators to a direct oxidase system of which the rate of reaction is limited by the supply of reducing reagent. Under such a scheme the difference between the rates of metabolism of glutamic and aspartic acids and of glycine and alanine would be explicable on the basis of the dehydrogenases present in the tissues.



The observations recorded in this paper are set forth in the following scheme for direct comparison with the scheme of Boswell (1945).

It has been assumed throughout that the conclusions of Boswell that the polyphenol oxidase is the major terminal oxidase in the slices of the potato tuber has found general acceptance and support. However, Schade and his co-workers (1948, 1949) have reached the conclusion from their work on slices and homogenates of the potato tuber that the terminal oxidases in the slices are two, cytochrome oxidase and an unknown oxidase referred to as X which oxidizes *p*-phenylene diamine. They take the view that polyphenol oxidase plays no part in the potato tuber system. The evidence which they present for the presence and function of cytochrome oxidase is completely adequate and there is no doubt that it is one of the direct oxidase systems in the tuber slices. The evidence from which they deduce that the polyphenol oxidase has no part as a terminal oxidase and on which they base their claim to discover enzyme X is defective owing to the absence of critical experiments, and their deductions are quite unsupported by their published results.

In accordance with the observations of all previous workers Schade found that the addition of catechol to the tissue resulted, with a certain exception which can be easily explained, in the stimulation of the oxygen uptake and in all cases this was followed by a rapid fall in the rate of oxygen uptake to some value 30–50 per cent. of the pre-addition value; further, that when a certain quantity of catechol was added the oxidase was completely inhibited and further additions had no effect. Since after overnight washing following the addition of catechol the residual oxygen uptake disappears entirely, Schade dismisses this oxygen uptake as due to the enzymatic oxidation of the catechol by non-viable slice tissue at a rate which diminishes with time. It is probable that had he measured carbon dioxide output as well as oxygen uptake he would have applied some adjective other than 'non-viable' to the tissue. That the residual respiration disappears during the overnight washing is hardly unexpected; in fact, it would have been surprising if the complete inhibition of a substantial part of the respiratory activity with the formation in excess in the tissue of such powerful oxidizing agents as quinones had not disorganized the highly integrated respiratory mechanism with the disappearance of respiration, leaving intact the activity of the oxidases as isolated systems, particularly since it is well known that the dehydrogenases in plant tissue are very sensitive to disorganization. On these results Schade considers that catechol acts as a tissue poison rather than as a specific inhibitor despite his own evidence of the specific inhibition of the oxidase. The distinction between the two effects is difficult to draw since fundamentally any tissue poison must inhibit some part of the respiratory mechanism and it is unimportant whether it does so by acting specifically upon some chemical system or by physical action upon the colloidal complexes. While taking account of the inhibitory action of catechol, Schade makes no reference to the results obtained using non-toxic water-soluble polyphenols such as caffeic acid which stimulate both oxygen uptake

and carbon dioxide output without any subsequent inhibition. Nor does he consider the relation of polyphenols to amino-acid metabolism.

Schade attempts to confirm his view that polyphenol oxidase has no part in slice respiration and to demonstrate the presence of the cytochrome oxidase by the use of carbon monoxide. As far as the cytochrome oxidase is concerned the light-reversible inhibition is adequate evidence for its presence, but the conclusions drawn on the non-participation in respiration of the polyphenol oxidase based upon the absence of any light-insensitive carbon monoxide inhibition under the experimental conditions used are wholly without value. For example, detailed results are given to show that in a mixture of 5 per cent. oxygen and 95 per cent. carbon monoxide the inhibition of the respiration rate is wholly reversible by light and cannot therefore be due to polyphenol oxidase. Since other data in the paper show that in 5 per cent. oxygen the rate of respiration is little more than 60 per cent. of the rate in air, all that the results really show is that this residual respiration in 5 per cent. oxgyen does not involve the polyphenol oxidase system. They do not permit any conclusion to be drawn about the nature of the oxidase system involved in that part of the respiration rate which was inhibited by the low oxygen pressure. Observations using a mixture of 20 per cent. oxygen and 80 per cent. carbon monoxide which are clearly of vital importance are very scantily reported. Under these conditions inhibition occurred only occasionally, and when it did so it never exceeded 20 per cent. and was light-reversible. In so far as these results show anything they show the participation of cytochrome oxidase. In view of the irregular occurrence of the inhibition to even a minor degree, it would appear more probable that the failure to demonstrate a light-insensitive inhibition is due to the fact that the concentration of carbon monoxide in the presence of 20 per cent. oxygen is insufficient to inactivate the polyphenol oxidase to the point at which the concentration of the free enzyme limits the rate of respiration, rather than to the non-participation of this enzyme as a terminal oxidase in the respiratory mechanism. Since data in the paper show that polyphenol oxidase is present in homogenates in concentrations such that the oxygen uptake due to its activity is 35 times that due to the cytochrome oxidase, it is to be expected that experimental conditions which only occasionally are sufficient to inhibit the cytochrome oxidase to the point at which the respiration rate is reduced are unlikely to reduce the polyphenol oxidase activity to a similar point.

From the great sensitivity of the rate of tissue respiration to reductions in the partial pressure of oxygen Schade postulated that in addition to the cytochrome oxidase there was present another oxidase which he designated X. This enzyme was regarded as responsible for the oxidation of *p*-phenylene diamine added to the tissue homogenates, since it is known that this substrate is not oxidized by polyphenol oxidase. What was overlooked was the fact that a tissue homogenate was not a pure preparation of the oxidase but a mixture of this enzyme with among other things the naturally occurring polyphenol of the potato tuber. Under the experimental conditions the polyphenol will be

oxidized to a quinone which oxidizes *p*-phenylene diamine by a purely chemical reaction. Enzyme X which in the homogenate oxidizes *p*-phenylene diamine is therefore none other than polyphenol-oxidase plus a polyphenol, forming a powerful redox system, the *p*-phenylene diamine acting as an H-donator to the quinone, reducing it to the *o*-dihydroxy state when it is available for re-oxidation by the oxidase. This enzyme X is described as being insensitive to carbon monoxide. When the data are examined it is found that the experiments were carried out using a gas mixture of 5 per cent. oxygen and 95 per cent. carbon monoxide in which the polyphenol oxidase would be relatively inactive, and even so a 20–25 per cent. inhibition occurred which was light-insensitive. Enzyme X is markedly sensitive to cyanide and to azide, which indicates a heavy metal in the enzyme structure.

These results indicate that enzyme X is a crude preparation of polyphenol oxidase and that Schade's observations, far from proving that this oxidase is not one of the terminal oxidases of potato tuber slices, provide additional confirmatory evidence that it is the principal terminal oxidase.

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Studies in the Genus *Elaphoglossum* Schott

I. Stelar Structure in Relation to Habit

BY

P. R. BELL

(*Botany Department, University College, London*)

With nine Figures in the Text

ABSTRACT

The habit is described of a number of species of *Elaphoglossum* collected in Jamaica. Species were present with creeping horizontal rhizomes, shortly creeping or ascendent rhizomes, and one species with an ascendent or upright rhizome.

The stelar structure of these rhizomes is described and it is shown that it bears a relation to the habit of the rhizome.

A preliminary examination of the apices has shown that the symmetry of the rhizome is determined in the initiation of the primordial tissues; it is not secondarily induced by the habit of the rhizome.

The occurrence of dormant buds is noted in all the species examined.

ELAPHOGLOSSUM (Schott, 1834) comprises a genus of Acrostichoid ferns, many of which have also been included in the genus *Acrostichum* itself. As a genus it is remarkable for the uniformity of the frond which, with few exceptions, is undivided and ovate-lanceolate or lanceolate in shape, both in the sterile and fertile state. The genus is more or less confined to the temperate mountain regions of the tropics, where some species ascend to 3,500 metres and higher. In addition, there are a few species in outlying islands such as Tristan da Cunha, Amsterdam Island, and the Azores. The general distribution of the genus is shown in Fig. 1, which is compiled from Christ (1900). It is interesting to note here that, although reaching as far south as Patagonia and south Chile, *Elaphoglossum* has nevertheless no representatives in the islands of Juan Fernandez which have an otherwise very rich flora of ferns, including the important endemic *Thyrsopteris elegans*. The northern limit of the genus is reached in the Azores, where a single species, *E. hirtum* (Swartz) C. Ch., grows in vegetable debris on the branches of trees and on rocks in the Juniper forest at an altitude of 1,200 to 1,500 metres (Boatman and Marler, 1949).

The representation of the genus in the Old World is very poor compared with that in the New. The greatest number of species occur in the Andean and sub-Andean regions, from Colombia and Venezuela to Bolivia and Brazil. These Andean species show a wide range of adaptation in relation to their habitat. The species of high altitudes are of lowly stature and usually possess a thick investment of imbricate scales. Those growing on the lower slopes are

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usually larger, with laxer fronds and scales that are often early deciduous, leaving the mature frond almost glabrous.

The most recent comprehensive systematic account of the genus is by Christ (1900), although since that time numerous new species have been described. The small variation in external morphology within the genus renders the taxonomic problem very difficult and the differences between the species, which lie principally in the form of the scales, are both critical and minute. The present state of the systematics of *Elaphoglossum* is far from

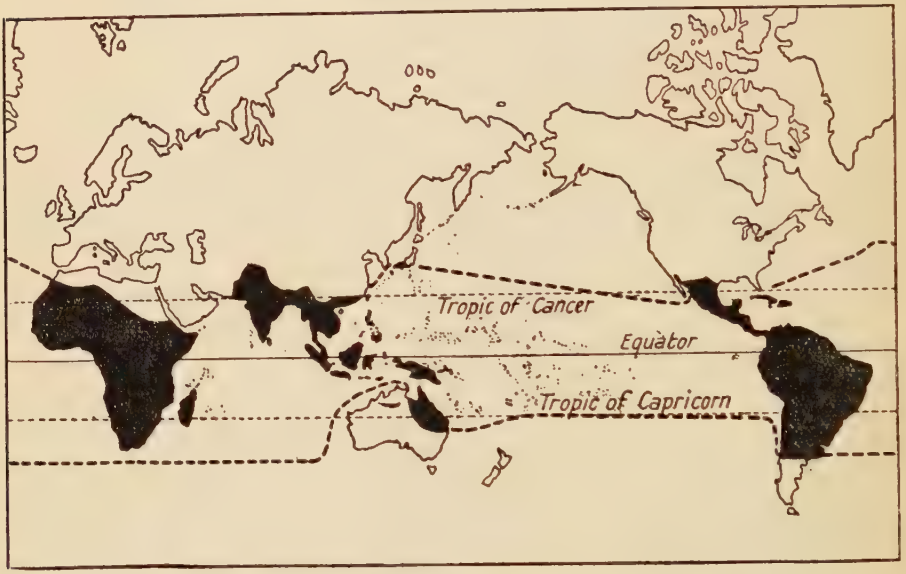

FIG. 1. *Elaphoglossum* Schott. Distribution of the genus.

satisfactory, but it is difficult to see how any substantial advance can be made until there is a clearer knowledge of the phylogenetic trends within the genus. This cannot be achieved by a study of the external morphology alone.

MATERIAL

The material that has been used in the present investigations was collected in the Blue Mountains of Jamaica in 1939 by H. Hamshaw Thomas and W. R. Philipson. The author is indebted to Dr. Thomas for passing on to him the preserved material and to Dr. Philipson and the British Museum (Natural History) for the loan of herbarium specimens. The preserved material, with the exception of *E. chartaceum*, was named by matching with herbarium specimens made at the time of collection and subsequently determined by W. R. Maxon. As there was no dried specimen of *E. chartaceum* this plant was named from other herbarium material. Nine species were represented in the collection, namely, *E. latifolium* (Swartz) J. Sm., *E. tectum* (H. and B.) Moore, *E. Eggersii* (Baker) Christ, *E. Sellowianum* (Presl) Moore, *E. chartaceum* (Baker)

C. Ch., *E. muscosum* (Swartz) Moore, *E. hirtum* (Swartz) C. Ch., *E. pallidum* (Baker) C. Ch., and *E. villosum* (Swartz) J. Sm.

Habit of the Rhizome

All the species investigated were collected in the Rain Forest Zone at an altitude of from 1,350 to 1,800 metres. The general habitat was vegetable

Fig. 2. *Elaphoglossum latifolium.* Portion of the rhizome with the scales removed; *a*, slightly elevated ridge of parenchymatous tissue on the dorsal side of the insertion of the petiole; *b*, bud on the abaxial side of the petiole; *r*, root; *r'*, root in association with bud; *s*, swelling in the petiole, about 2 cms. above the rhizome, at which the old fronds disarticulate.

debris either on banks, rotting logs, or branches of forest trees. In some species, such as *E. latifolium*, the petiolcs of the fronds appear always to ascend. In others, such as *E. villosum*, the fronds may be pendent or the plant may grow on the underside of a branch and the fronds ascend only at the tips. All species possess a rhizome, varying from 1 cm. to 0·5 cm. or even less in diameter, according to the species.

The habitat of the rhizome of *E. latifolium*, after removal of the close covering of scales from both the rhizome and petiolar bases, is shown in Fig. 2. The rhizome creeps horizontally and bears fronds in two rows, one on each side of the median line with the insertions in each row arranged alternately. The distance between the insertions in one row varies between 1·5 and 2 cm. On the abaxial side of each petiolar base is a conspicuous

dormant bud. None of these buds was observed to have undergone any prolonged development, although it is probable that destruction of the main apex would be followed by the development of one or more of these buds. Two or three small roots arranged linearly in an obliquely ventral direction are frequently seen close behind the bud. One of these series is visible in Fig. 2. Other than these small roots associated with buds, the bearing of roots is confined to the ventral surface of the rhizome.

These features of the rhizome of *E. latifolium* are also found in *E. Sellowianum*, *E. tectum*, *E. Eggersii*, and *E. chartaceum*, all of which have horizontal creeping rhizomes. Species differ in the diameter of the rhizome and the distance between the insertions of the petioles, but the general picture remains unaltered.

In *E. muscosum*, *E. hirtum*, and *E. pallidum* the rhizome is not longly creeping, but is shortly horizontal or obliquely ascendent. It is never completely upright. The fronds in these species are not borne in two rows, but are crowded together in several rows on the dorsal side of the rhizome. The insertions tend to form diagonal as well as longitudinal rows, but with no clear regularity. Buds are found only on the abaxial side of those petioles nearest the ventral surface of the rhizome. These buds are much less conspicuous than those found in the previous group of species, but in one large specimen of *E. hirtum*, with a rhizome about 12 cm. long, two of these buds, both about 9 cm. from the apex, had developed into short branches. The parent rhizome was hard and brittle in this region and presumably dead. Its fronds were confined to a distance of about 3 cm. behind the apex; only necrotic leaf bases were present along the rest of the rhizome. The short branches had reached a diameter about half that of the parent rhizome and the fronds upon them about one-quarter of the mature size. With progressive decay of the parent rhizome, these outgrowing buds would no doubt remain as independent plants. As in the former group of species, buds may have two or three rootlets behind them on the parent rhizome, but otherwise roots are again confined to the ventral surface.

In the last species, *E. villosum*, the rhizome is either obliquely ascendent or even completely upright. The fronds are crowded together and inserted all around the rhizome, although there is no evident phyllotaxy. The fronds are also often secund, an effect probably due to the position in which the plant grew. Minute dormant buds are present on the abaxial side of the bases of some of the petioles. These buds are very difficult to detect externally and have never been found to have undergone any development. The roots appear between the petioles and from the bases of the insertions; they are not restricted to any particular part of the rhizome, which is thus devoid of any recognizable dorsal and ventral surfaces.

Stelar Structure

A reconstruction of the stele of *E. latifolium*, built up from a series of transverse sections, is shown in Fig. 3. It consists essentially of a broad

ventral meristele and a smaller, median, dorsal meristele. These two meristeles are joined at intervals along their margins by vascular bridges. A well-developed bud trace leaves the margin of the ventral meristele close to the insertion of the bridge. This bud trace frequently gives rise to small root traces

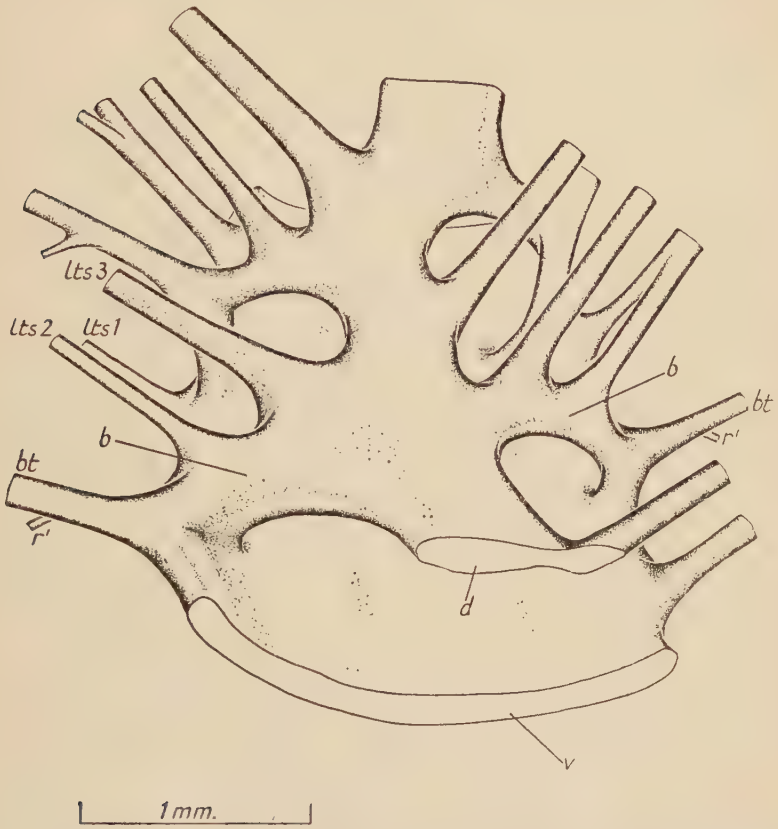

FIG. 3. *Elaphoglossum latifolium.* Reconstruction of the stele; *v*, ventral meristele; *d*, dorsal meristele; *b*, bridge connecting the ventral and dorsal meristeles; *bt*, bud trace; *lts*, strand of the leaf trace; *r'*, root in association with bud trace.

in its passage through the cortex which run into the linearly arranged rootlets behind the bud. The strands of the leaf trace, three to five in number at its origin, arise both from the bridge and from the margins of the ventral and dorsal meristeles. The space between one marginal bridge and the next constitutes the leaf gap. Root traces, other than those associated with the bud trace, arise only from the ventral surface of the ventral meristele.

All those species examined which possess a horizontal rhizome bearing two rows of leaves have shown this type of stele. The difference between the stele of one species and that of another has been one of proportion only; there has been no change in the essential plan of construction.

In those species with an horizontal or obliquely ascending rhizome bearing more than two rows of leaves, the stele is more complex. A reconstruction of the stele of *E. muscosum* is shown in Fig. 4. The general pattern seen in *E. latifolium* is still recognizable. The ventral and dorsal meristeles, the marginal bridges, and the bud traces arising from the margins of the ventral

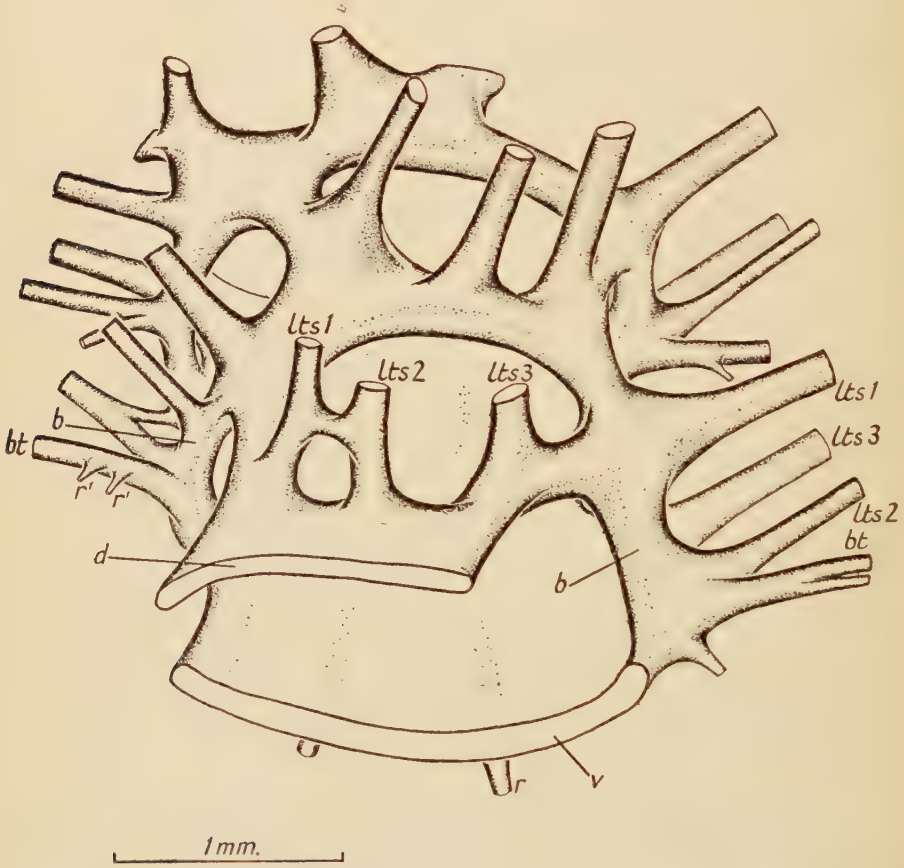

FIG. 4. *Elaphoglossum muscosum*. Reconstruction of the stele; *v*, ventral meristele; *d*, dorsal meristele; *b*, bridge connecting the ventral and dorsal meristeles; *bt*, bud trace; *lts*, strand of the leaf trace; *r*, root; *r'*, root in association with bud trace.

meristele are all present. The complication arises in the region of the dorsal meristele, which appears to have been broken up by the insertion of the traces of the additional rows of leaves. The departure of these traces leaves a considerable leaf gap, but, in spite of this, the dorsal meristele more or less retains its identity. No bud traces have ever been observed to arise from the dorsal meristele, nor any buds found in association with the bases of the petioles into which the traces from the dorsal region enter.

A stele precisely similar to that seen in *E. muscosum* was found in *E. hirtum*,

but *E. pallidum* has a stele which, although closely related, is yet more complex. A transverse section of this stele is shown in Fig. 5. It is seen that the ventral meristele is still clearly present, but the stele in the region of the dorsal meristele is now very much more dissected by the insertion of leaf traces. The general pattern, however, remains recognizable and the

FIG. 5. *Elaphoglossum pallidum.* Transverse section of the stele; *v*, ventral meristele; *bt*, bud trace; *lts*, strand of the leaf trace; *r*, root trace; *r'*, root trace in association with bud trace.

bud traces are still found solely in association with the margin of the ventral meristele.

In all these species considered so far there is a clear dorsiventrality in the stelar structure of the rhizome. The simplest type is seen in *E. latifolium*. The complex pattern seen in *E. pallidum* is attained by progressive elaboration of the dorsal part of the stele. This increasing dissection of the dorsal part is accompanied by a certain contraction of the ventral meristele and also of the degree of development of the bud trace, which is markedly less well developed

in *E. pallidum* than in *E. latifolium*. There is seen in the steles of these species a tendency towards radial symmetry and dictyostely, but this tendency is far from completion.

In *E. villosum* the stelar structure is quite different. A reconstruction is shown in Fig. 6. There is no trace of dorsiventrality in the stele; it is, on the contrary, radially symmetrical and dictyostelic. Each leaf trace consists of

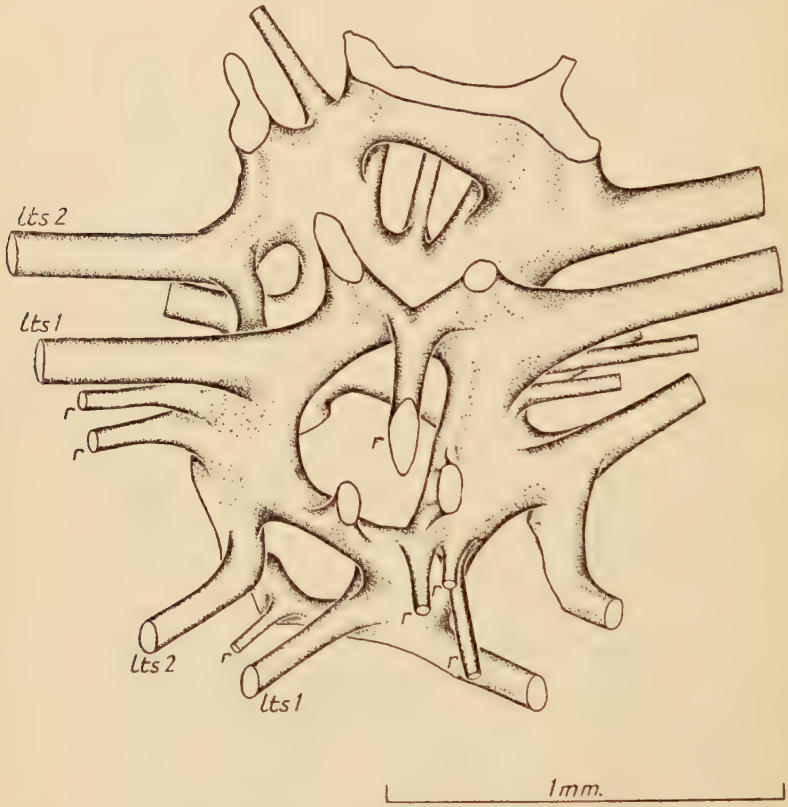

Fig. 6. *Elaphoglossum villosum*. Reconstruction of the stele; *lts*, strand of the leaf trace; *r*, root trace.

two strands which depart from the basal margin of a relatively large leaf gap. The root traces are inserted below the sinus at the base of the leaf gap. The bud trace, where present, also departs from this region, immediately anterior to the uppermost root trace. There are no bud traces shown in Fig. 6 as it was impossible to recognize them in the series of sections from which this reconstruction was made, but, in a later series, bud traces were observed in association with three successive leaf traces (Fig. 7). The evidence upon which these traces, which are extremely tenuous and poorly differentiated, are identified as belonging to buds will be presented later in a comparative consideration of the anatomy and course of the bud trace.

Although, as has been seen, the structure of the stele bears a relationship to the habit of the rhizome, it is evident from an examination of the apices and their subsequent development that the dorsiventral stele is not a direct modification brought about by habit. The apices of these ferns are quite striking in their flat appearance. Well-developed fronds occur only a very short distance behind the apex and fully differentiated roots may emerge

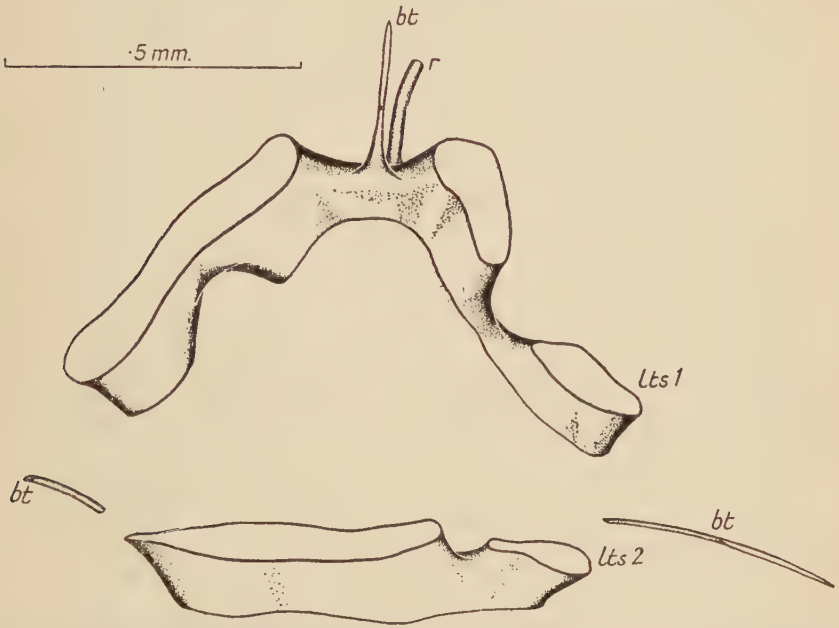

FIG. 7. *Elaphoglossum villosum.* Reconstruction of a small part of the stele to show bud traces in association with three successive leaf traces; *bt*, bud trace; *lts*, strand of the leaf trace; *r*, root trace.

almost up to point where the transverse curvature of the apex begins. Internally, although differentiation of the xylem begins very close behind the apex, there is very little spiral thickening of the tracheides; it is almost wholly annular or scalariform. In the mature tissues, too, there is only occasionally seen a protoxylem tracheide with an extended spiral or ruptured annular thickening. Both the external morphology and the internal anatomy point to the absence in the apex of any region of rapid cellular extension.

Apices have been examined of all the species except *E. Sellowianum*, where no material was present. Owing to their long preservation in spirit these apices had become very hard and permeated with resin. It was not possible to embed them in wax by normal methods. Rather than experiment with so little available material it was decided to examine them by freehand sections. In every instance a single apical cell was seen at the tip of a small, but definite, apical cone. But the general symmetry of the apex was found to vary in relation to the series of stelar forms already demonstrated. A diagram of a

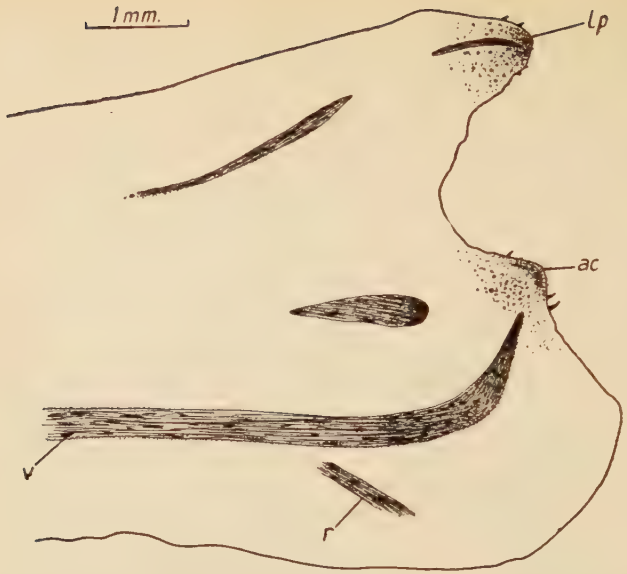

FIG. 8. *Elaphoglossum tectum.* Diagram of a median vertical longitudinal section through the apex of the rhizome; *lp*, leaf primordium; *ac*, apical cone; *v*, ventral meristele; *r*, root trace.

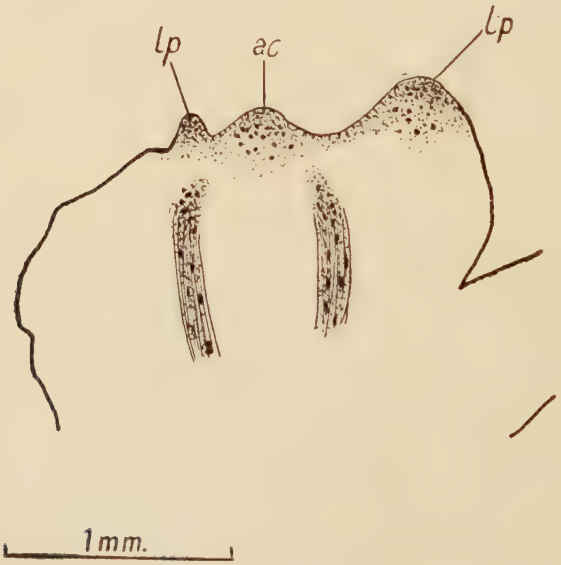

FIG. 9. *Elaphoglossum villosum.* Diagram of a median longitudinal section through the apex; *lp*, leaf primordium; *ac*, apical cone.

median vertical section of the apex of *E. tectum*, a species with a long creeping rhizome bearing two rows of leaves, is shown in Fig. 8. Here the initial tissues themselves are disposed in bilateral symmetry about the median vertical plane. The leaf primordia are confined to the dorsal side of the apical region. On the ventral side of the apex there is a marked forward protrusion of parenchymatous tissue. This protrusion possesses no area of pronounced meristematic activity and must result from a general inequality of growth about the apical meristem. The apical cone actually lies behind this ventral protrusion.

All species possessing a dorsiventral stelar structure have shown this type of bilaterally symmetrical apex. In *E. muscosum*, *E. hirtum*, and *E. pallidum*, where the rhizome bears several rows of leaves, the ventral protrusion is much less marked and may equal or only slightly exceed the apical cone.

E. villosum differs markedly from the preceding species. A diagram of a median longitudinal section of the apex is shown in Fig. 9. Here there is quite normal radial symmetry. The only structures exceeding the apical cone are the developing leaf primordia. There is nothing resembling the ventral protrusion seen in the other species.

The significance of the trends in stelar form here described and their relation to the phylogenetic trends within the genus will be considered at a later stage when all the relevant evidence is available. In the present paper the idea of progression has been used in a figurative sense and without any phylogenetic connotation.

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The Suspensor Haustoria of Some Species of *Crotalaria* Linn.

BY

M. ANANTASWAMY RAU

(*Department of Botany, University of Mysore, Mysore City, India*)

With twenty-two Figures in the Text

ABSTRACT

The structure and function of the suspensor has been studied in eight species of *Crotalaria*, in all of which it shows a differentiation into an upper and a lower part. The peripheral cells of the lower part project out and act as haustorial structures for the absorption of nutrition from the surrounding endosperm. In *C. retusa* and *C. striata* these processes are very long and tubular attacking the surrounding endosperm and nucellar tissues and extending even into the integumental layers. Remains of the suspensor are recognizable even in the mature seed. The cells of the suspensor possess chloroplasts so that this organ also has a photosynthetic function in addition to its haustorial activity.

THE genus *Crotalaria*, belonging to the tribe Genistae of the Papilionaceae, comprises nearly 350 species distributed in tropical and sub-tropical regions. Of these no less than seventy-five occur in south India alone (Gamble, 1915). Cook (1924) studied the embryology of *C. sagittalis* and Samal (1937) of *C. juncea*, well known for its fibre. Both have reported the presence of a massive suspensor in the embryo. The present investigation, which was undertaken to make a comparative study of the nature and haustorial activity of the suspensor in this genus, deals with the following eight species: *C. laburnifolia* Linn., *C. verrucosa* Linn., *C. retusa* Linn., *C. striata* DC., *C. orixensis* Rottl., *C. albida* Heyne., *C. paniculata* Willd., and *C. biflora* Linn.

The material was collected in Mysore and fixed in formalin-acetic-alcohol. The sections were stained in Heidenhain's iron-alum haematoxylin with eosin as a counterstain. In later stages it was found more advantageous to dissect out the embryo and prepare whole mounts, which gave a three-dimensional picture of this organ and showed the chloroplasts in the suspensor cells in their natural colour. The seed is cut through the micropyle into two unequal halves so as to avoid injuring any portion of the embryo. In the larger half the embryo can be seen as a green slender structure which is easily picked out with a needle. In an aqueous whole mount it is a remarkable sight to observe the suspensor wriggling about on account of the lateral protuberances of the peripheral cells stretching out after their release from the surrounding tissue where they were all along held in position. This is particularly marked in

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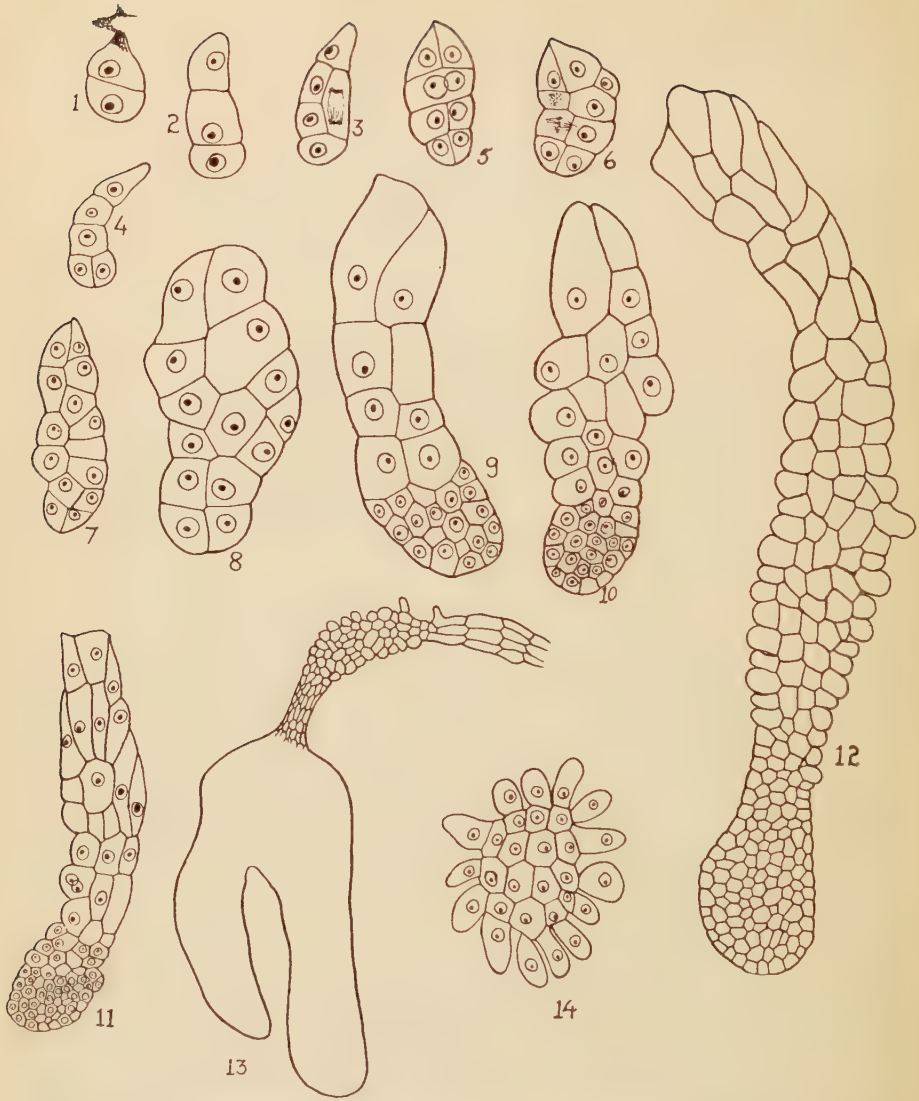

FIGS. 1–13. *C. laburnifolia*: Stages in the development of the embryo. Figs. 1–8. ×560. Figs. 9, 10. ×400. Figs. 11, 12. ×200. FIG. 13. ×80. FIG. 14. Cross-section through the suspensor. ×260.

those species in which the peripheral cells form long tubular processes. Stages older than the one shown in Fig. 11 were easily followed with the aid of whole mounts alone, some of which were made permanent by the usual methods of fixing, staining, and mounting in balsam.

Observations

The ovules are usually arranged in two rows on the marginal placenta. In some species the number of ovules is very small and in *C. paniculata* it is reduced to one or two. The ovules are mostly amphitropous and are sharply bent at the micropylar end. There are two integuments, of which the outer is more massive, particularly in the region of the micropyle. The inner integument is shorter and does not take part in the formation of the micropyle. It is made up of only two cell layers which also get crushed during the development of the seed so that the seed coat is formed only from the outer integument.

The fertilized egg divides transversely after a few endosperm nuclei have been formed (Fig. 1). As a rule the basal cell (the one near the micropyle) elongates and again divides by a transverse wall resulting in a three-celled proembryo (Fig. 2). The middle cell undergoes vertical and transverse divisions to give rise to a group of four cells (Fig. 3). At about the same time the terminal cell divides vertically (Fig. 4) and there is a similar division in the basal cell (Fig. 5). Hereafter, in the central region the divisions are mostly by oblique walls, but at the embryonal end they are more regular, so that an embryo-forming region (derived chiefly from the terminal cell) becomes more or less clearly demarcated from the suspensor-forming region (Figs. 6–11). The latter is itself roughly divisible into two regions, the upper consisting of greatly elongated or enlarged cells and the lower consisting of bulbous cells of which those towards the periphery project out to varying extents. This lower region of the suspensor is mostly the product of divisions of the middle cell of the three-celled proembryo. Fig. 14 shows a cross-section of the suspensor and gives an idea of its thickness. Cook (1924) reported a massive suspensor in *C. sagittalis*, but this appears to be much shorter than that of other species. In *C. juncea* (Samal, 1937) it is very long and shows the two regions clearly. The following variations in the organization and activity of the suspensor have been noticed in the species described here. In *C. albida* and *C. orixensis* the suspensor is comparatively short and the upper region shows slightly elongated cells with highly vacuolated cytoplasm. The lower region also contains enlarged cells of which the peripheral bulge out slightly and act as haustorial structures for the absorption of nutrition from the neighbouring endosperm tissue (Figs. 15, 16). In *C. verrucosa* (Fig. 17), *C. laburnifolia* (Figs. 12, 13), *C. paniculata* (Fig. 18), and *C. biflora* the suspensor is much longer. Its upper part consists of vertically elongated cells which seem to serve as haustorial structures for the absorption of nutritive materials from the nucellar tissue at the micropylar end. In the lower part the peripheral cells protrude out and absorb food from

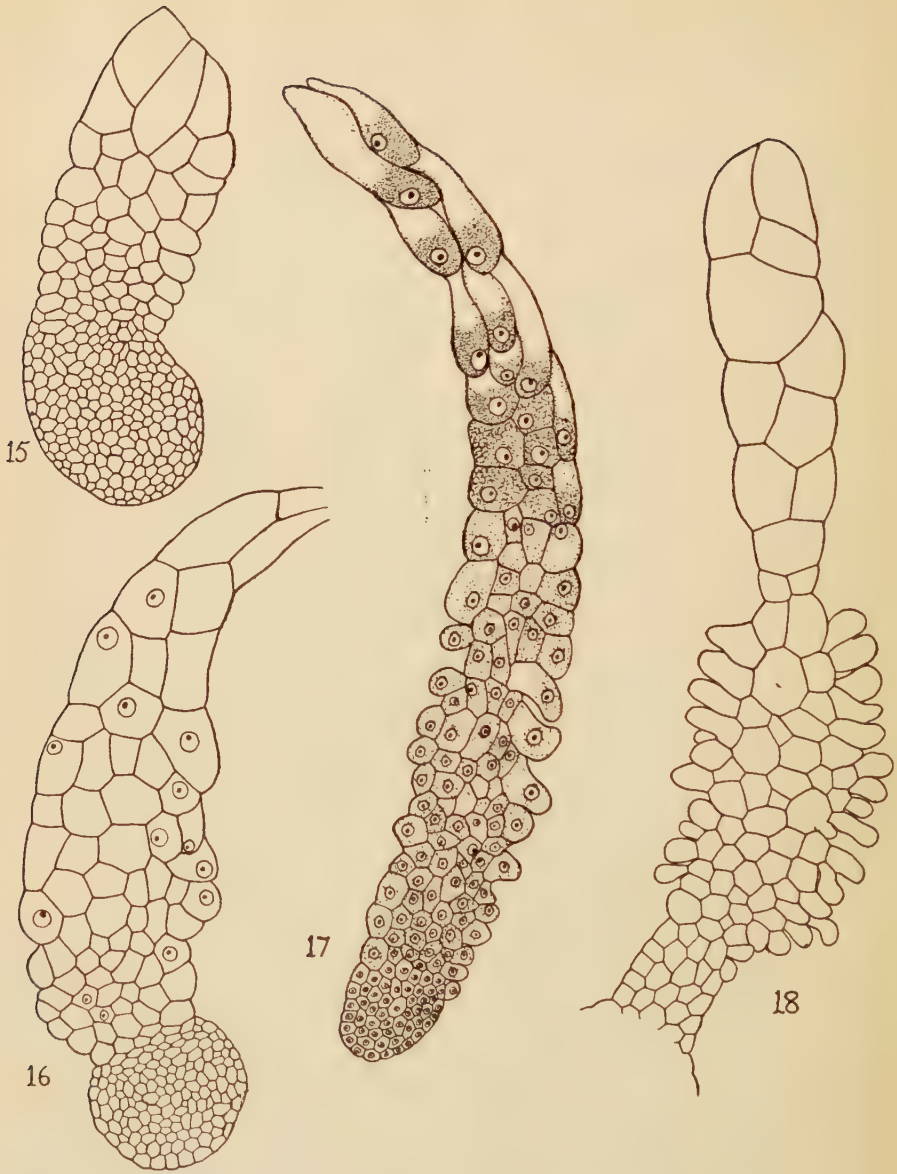

FIG. 15. *C. albida.* ×200. FIG. 16. *C. orixensis.* ×200. FIG. 17. *C. verrucosa.* ×160.
FIG. 18. *C. paniculata.* ×200.

the endosperm. Of all the species studied *C. retusa* and *C. striata* show the most pronounced haustorial activity of the suspensor. In the lower part the peripheral cells put forth tubular processes which encroach upon the endo-

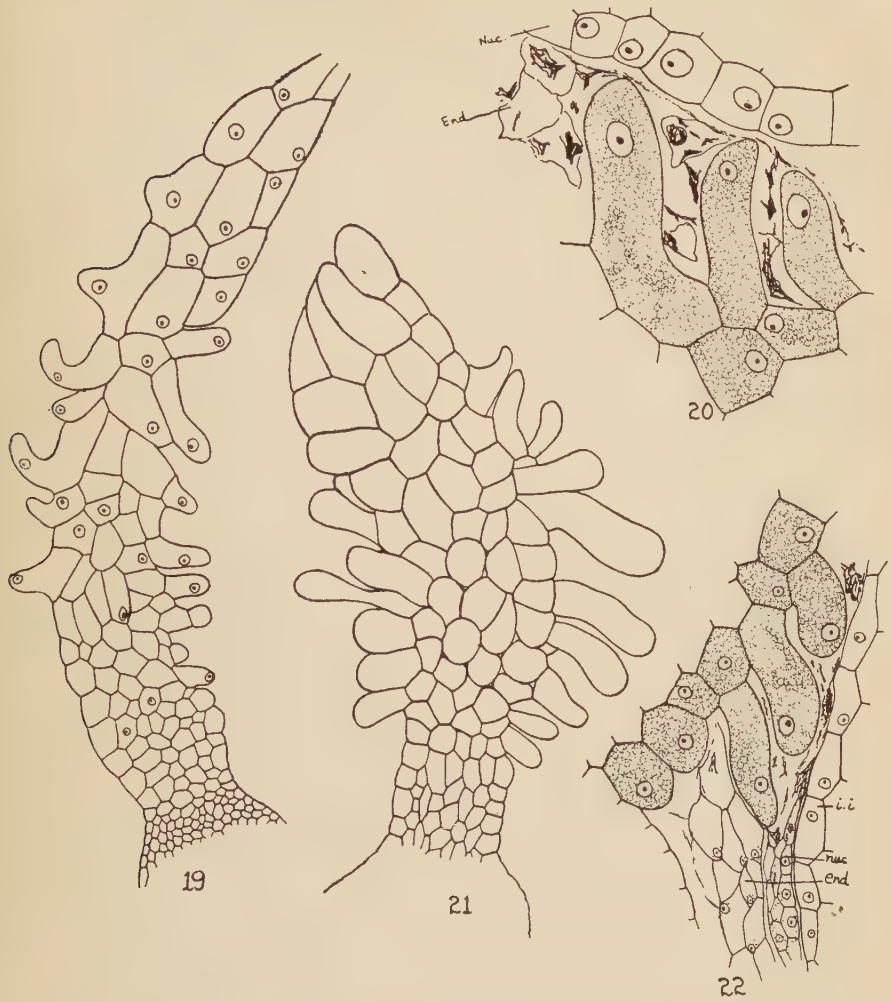

FIG. 19. L.S. of embryo of *C. retusa*. × 160. FIG. 20. More highly magnified view of the peripheral cells of the lower part of the suspensor showing their encroachment on the surrounding endosperm tissue. × 400. FIG. 21. L.S. of upper part of embryo of *C. striata*. × 200. FIG. 22. More highly magnified view of peripheral cells of the lower part of the suspensor attacking the endosperm and nucellar layers and reaching the inner layer of the integument. × 400.

sperm and then extend into the nucellar region (Figs. 20, 22). In *C. striata* (Figs. 21, 22) these tubular projections advance in every direction and reach even the integuments.

Another interesting feature, already referred to before, is the presence of numerous chloroplasts in the cells of the suspensor, so that it also serves as

a photosynthetic organ in addition to its haustorial activity. The presence of chlorophyll has been previously reported by Strassburger (1880) in the suspensor cells of a species of *Lupinus*.

As already stated, divisions in the lower region of the embryo are fairly regular and result in the formation of a massive group of cells which are small and densely cytoplasmic. The cotyledons and the other embryonal regions are differentiated from this in later stages. The suspensor is recognizable even in the mature seed.

Wall formation in the endosperm takes place when the embryo has reached an advanced stage in development. The walls are first laid in the micropylar region but later extend downwards. However, even in old stages some large free nuclei can be seen at the chalazal end. Most of the endosperm is used up when the seeds are mature and the embryo occupies the entire seed cavity.

I thank Professor P. Maheshwari of the Delhi University for kindly going through the manuscript and giving valuable suggestions.

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